EVALUATION OF ANTICONVULSANT AND ANALGESIC PROPERTIES OF ETHANOLIC EXTRACT OF *ELETTARIA CARDAMOMUM* SEEDS IN WISTAR ALBINO RATS

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

In partial fulfilment of the requirements for the award of the degree of

M.D. PHARMACOLOGY

Branch VI

APRIL 2017

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CERTIFICATE

This is to certify that this dissertation entitled "**Evaluation of Anticonvulsant and Analgesic properties of Ethanolic extract of *Elettaria cardamomum* Seeds in Wistar Albino rats**" is a bonafide record of the work done by **Dr. Arjun.G.Nair** under my guidance and supervision in the Department of Pharmacology during the period of his postgraduate study for **M.D. Pharmacology [Branch – VI]** from 2014-2017.

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I, Dr. Arjun.G.Nair. A hereby submit the dissertation entitled "**Evaluation of Anticonvulsant and Analgesic properties of Ethanolic extract of *Elettaria cardamomum* Seeds in Wistar Albino rats**" done in partial fulfilment of **M.D. Pharmacology [Branch-VI]** in Sree Mookambika Institute of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of Dr. Reneega Gangadhar.

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INTRODUCTION

1. Introduction

Traditional system of medicine has a very long history in prevention, diagnosis and treatment of various illnesses.[1] According to the reports from World Health Organization (WHO), eighty percent of the population in developing countries depends on plant derived medicines for the prevention and treatment of diseases.[2] These herbal medicines are time tested and already in clinical use, even though they do carry risks and many people think that all natural treatment is inherently safe. Despite widespread usage, the safety and efficacy of these drugs have not been evaluated in an evidence based manner.[3] Though India is a paramount of vast genetic, species and ecosystem diversity, we have only a small role in the International market of herbal drugs, owing to the lack of proper scientific information about their mechanism of action, efficacy and safety.[4] Therefore research in this area have to be strengthened up to prove their effectiveness .

Convulsions have been the most visual diagnostic signs for epilepsy. Epilepsy is considered to be a common neurological abnormality seen in medical practice. About 50 million people around the world have epilepsy of which more than 85% of cases are seen in developing countries. Annually, about two million new cases are reported worldwide.[5] The selection of anticonvulsant drugs will depend upon the type of epilepsy. Although there are several anticonvulsant drugs available, still the treatment of epilepsy is limited due to the potential adverse drug reactions. So there is a need for newer anticonvulsant drugs with more degree of compliance.[6]

Epilepsy is recognized as a "disease of lightening" as pointed out by J.S Jackson in the early 19th century. There are various theories in the pathogenesis of epilepsy and many drugs have been tried to decrease the altered neuronal discharge. Oxidative stress resulting from excessive free-radical release is one reason implicated in the initiation and progression of epilepsy. Therefore, antioxidant therapies aimed at reducing oxidative stress have received considerable attention in the past few years in the treatment of epilepsy.[7]

Pain is an unpleasant sensation that often accompanies certain pathological conditions often demanding effective treatment with analgesics. Non-steroidal Anti-Inflammatory Agents (NSAIDs) and centrally acting Opioids are the commonest drugs prescribed as analgesics. The NSAIDs which are usually used for mild to moderate pain is limited by their adverse effects, including gastrointestinal, renal and cardiac effects. Opioids are hypnotics with analgesic effect, considered as the mainstay in pain management including palliative care. Their use is checked by the adverse effects and abuse liability.[8] Search for newer effective analgesics is still a growing need in the management of pain. Oxidative stress is implicated in the pathophysiology of pain and there is an increase in the free radicals during painful stimulation. It has been reported that the antioxidants by decreasing the free radicals have significant nociceptive effect when they are used along with analgesic agents.[9,10]

According to our indigenous system of medicine, *Elettaria cardamomum* , popularly known as “Cardamom or Elaichi” is a herb found mostly in India , Pakistan , Burma and Sri Lanka having many medicinal properties. It belongs to the order Scitaminae and are commonly used to impart aroma and flavor to dishes and desserts.[11] Cardamom has the reputation of being used as a carminative, diuretic, cardio protective, abortifacient, expectorant and appetite stimulant. Patients with halitosis, anorexia, gastric irritation or burning sensation, weakness, bronchial asthma, hemorrhoids, renal stones, also benefit from this plant.[12] Several studies were done on this plant demonstrating its anticonvulsant, analgesic, antioxidant, sedative, bacterial, antiulcerogenic, antihypertensive, antispasmodic, hepatoprotective, antioxidant and antiplatelet properties.[13-19]

Based on the historical information, the present study is conducted to evaluate and validate the anticonvulsant and analgesic activities of Ethanolic seed extract of *Elettaria cardamomum* using different models in Wistar albino rats.

HYPOTHESIS

&

JUSTIFICATION

2.1 Hypothesis

Ethanolic extract of Elettaria cardamomum seeds have anticonvulsant and analgesic properties.

2.2 Justification

Excessive production of oxidative free radicals leading to neuronal hyper excitability have been implicated in the pathogenesis of a considerable range of various pathophysiological conditions like epilepsy and pain. Experimental studies suggest that oxidative stress is a contributing factor to the onset and evolution of epilepsy. Antioxidants are endogenous or exogenous substances which act by reducing free radical formation, free radical scavenging or removal of free oxygen.[20] Oxidative stress and mitochondrial dysfunction is implicated in neuronal death, hence has important role in the onset and maintenance of seizures. It was shown that partial prevention of induced seizures is possible with the use of antioxidants.[21] Currently available analgesics and antiepileptic drugs, have limitations such as toxicity, teratogenicity, drug resistance, and significant pharmacokinetic interactions. Hence, antioxidants may be an important adjunct to conventional therapies and also they serve as a direction for development of novel antiepileptic drugs.[22]

Cardamom seeds are found to have antioxidant potential. Moreover they have a great significance as a phytomedicine in the treatment of epilepsy in our indigenous system of medicine.[18] So in view of these

facts, a proper documentation of the anticonvulsant property of *Elettaria cardamomum* is not available. Analgesic property of *Elettaria cardamomum* was demonstrated in mice using acetic acid induced writhing test. Therefore more number of studies is needed to validate its anticonvulsant and analgesic properties.

AIMS

&

OBJECTIVES

3. Aims and Objectives:

i. To evaluate the Anticonvulsant property of Ethanolic extract of *Elettaria cardamomum* seeds on electrically-induced seizure model using maximal electroshock and chemically-induced seizure model using pentelenetetrazole in Wistar Albino rats.

ii. To evaluate the Analgesic property of Ethanolic extract of *Elettaria cardamomum* seeds on thermal stimuli induced pain model using tail warm water immersion test and acetic acid induced pain model using writhing test in Wistar Albino rats.

REVIEW OF LITERATURE

4. Review of Literature

4.1.1 Pharmacognosy of *Elettaria cardamomum*

The American Society of Pharmacognosy (ASP) defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." [23] At the beginning of the 20th century, pharmacognosy was used to define the branch of medicine which deals with drugs in their crude or unprepared, form. The advent of the 21st century brought a renaissance of pharmacognosy and its conventional botanical approach has been broadened up to molecular level. Various disciples that come under pharmacognosy are medical ethnobotany, ethnopharmacology, phytotherapy, phytochemistry, zoo pharmacognosy. Pharmacognosy is now considered as an essential domain of modern pharmaceutical science.[24]

Historically known as the “Queen of all Spices”, cardamom has been used in India since ancient times. The pioneer reference to cardamom was dated back to the Vedic period (3000 BC), mentioning its use in helping digestion in Ayurvedic books. Cardamom was used by Egyptians, as a mouth freshener. The scientific name of cardamom plant is *Elettaria cardamomum* and it comes under family Zingiberceae. The plant exhibits good diversity in geography, size and percentage of the various chemical components of the volatile oil in the seeds.[25]

Zingiberacea or the ginger family which consists of fifty two genera, is one of the most recognized families of the order *Scitamineae*. Members of the family are small to large herbaceous plants with distichous leaves with basal sheaths that overlap to form a pseudostem. *Elettaria* is a genus of plants in this family, consisting of eleven species which are found in various tropical countries around the world. It is native to India, Sri Lanka, Borneo, Sumatra and Malaysia, though some species are naturalized elsewhere.[26]

Elettaria cardamomum (L.) Maton (synonym - *Amomum cardamomum* L.) is one of the medicinally important plants native to Southern part of India. It is the most common of the species whose seeds are used in seasoning food. Cardamom is used in Indian cuisine as spice for gravy, coffee, cakes, bread, and flavoring sweet dishes and drinks. It is also chewed as a mouth freshener. They are used in Ayurvedic medicine for various disorders of gastrointestinal, cardiovascular and nervous system. Cardamom is one the commonly used major constituent in many classical formulations of Ayurveda. It is the second most expensive spice in the world by weight, with only saffron being more expensive than it is.[27] The preliminary phytochemical analysis of ethanolic extract of Elettaria cardamomum showed the presence of alkaloids, saponins, flavonoids, tannins and phenolic compounds, terpenoids and phytosterols, fixed oils and fatty acid, carbohydrates and proteins.[28] The experimental studies proved beyond doubt that the plant possess many

medicinal properties like gastroprotective, antioxidant, sedative, antimicrobial etc.[12]

4.1.1 Taxonomical hierarchy

Kingdom	:	Plantae
Subkingdom	:	Viridiplantae
Infrakingdom	:	Streptophyta
Superdivision	:	Embryophyta
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Superorder	:	Lilianae
Order	:	Zingiberales
Family	:	Zingiberaceae
Genus	:	*Elettaria*
Species	:	*Elettaria cardamomum* (L.) Maton[29]

4.1.2 Habitat and distribution

The natural habitat of *Elettaria cardamomum* is the evergreen forests of Western Ghats in India. It is grown in the areas where the annual rainfall ranges from 1500 to 4000 mm, with a temperature range of 10^0C to

35^0C and an altitude of 600 to 1200 m above mean sea level. Cardamom grows luxuriantly in humus rich soils, which are generally acidic in nature with a pH range of 5.5-6.5. It is also widely seen in the tropical regions of Malaysia and Costa Rica.[30]

4.1.3 Growth and propagation

E.cardamomum grows well either in full sun or partial shade. It requires soil rich in organic matter with adequate moisture. Cardamom is mainly propagated by seeds and vegetative propagation using rhizomes. In the commercial cultivation of cardamom, micropropagation using tissue culture technique can also be practiced.[31]

4.1.4 Morphological features of *Elettaria cardamomum*

Elettaria cardamomum is an aromatic herbaceous perennial plant. From the underground stem there arises many stems that are about 5 to 18 feet in height that bears long leaves. The leaves are alternate in two ranks, linear-lanceolate, 40–60 cm (16–24 in) long, with a long pointed tip. Flowers are present in racemes presentation which is 2 to 4 feet in height. The flowers are white to lilac or pale violet, produced in a loose spike 30–60 cm (12–24 in) long. The fruit is a wrinkled three-sided pod 1–2 cm (0.39–0.79 in) long, containing several black and brown seeds. Fruit is triangular which is of yellow to green in color. There are 15 to 20 very hard angular seeds inside the fruit.[32]

4.1.4.1 Macroscopic features of *Elettaria cardamomum* seed

The fruit encompasses a capsule with three cells comprising of plenty of seeds. The seeds are seen mostly in groups of two to seven. The individual seeds are shaped ovoid with three asymmetrical walls. The dorsal surface is convex and fissured longitudinally on each side. The volume of each side is roughly around three cubic millimeters. They are dark reddish-brown, asymmetrically angular, grooved and bounded in a thin, membranous, brown cover. They are rough, tuberculated and strongly adheres to the outer cover. Cut-section of seed, it displays a thin brown seed-coat, a large white perisperm and a central, slightly greenish endosperm enclosing the embryo.[33]

4.1.4.2 Microscopic features of *Elettaria cardamomum* seed

The seed fragments on microscopy showed mainly two types of cells namely seed cells and stone cells. Microscopic examination of the seed cells revealed the presence of numerous starch granules each of approximately 0.003 mm in diameter. Each stone cell is about 0.03 mm in diameter, polygonal in shape and appears dark-brown color. The powdered seeds exhibit abundant, minute, angular starch grains, often compacted into masses. The epidermal cell cytoplasm contains calcium oxalate crystals, wheras the endosperm cells are rich in oil, starch and proteinoid granules.[34]

4.1.5 Phytochemistry

Shetty et al.[28] performed phytochemical screening of ethanolic extract of *Elettaria cardamomum* fruits. The preliminary phytochemical analysis of ethanolic extract of *Elettaria cardamomum* showed the presence of alkaloids, saponins, flavonoids, tannins and phenolic compounds, terpenoids, phytosterols, fixed oils, fatty acid, carbohydrates and proteins. This explains the important pharmacological activities shown by the plant like antioxidant, gastroprotective, antihypertensive, antispasmodic, antibacterial, antiplatelet and anticancer properties in various animal studies. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the antioxidant activity of cardamom. Saponins have been reported to have antimicrobial properties, cholesterol lowering, anti-inflammatory, hypotensive and they act as important precursor for steroidal substances. These steroidal substances claimed to have wide range of pharmacological activities. The terpenoids and sesquiterpenes present in the oils and are found to exhibit anti-inflammatory and antimicrobial effects.

Kumar et al.[35] obtained cardamom oil through steam distillation by continuous extraction soxhlet method. The extract was then screened for the presence of alkaloids, carbohydrates, proteins, steroids, glycosides and terpenoid compounds. The phytochemical analysis showed alkaloids, glycosidic compounds, carbohydrates, steroids, tannins, terpenoids and

phenolics are present. Column chromatographic separation using toluene yielded eleven compounds f1 to f11. Spectrophotometric analysis of cardamom showed maximum absorption at wavelength 253 nm which could be attributed to benzene.

Sharma et al.[36] reported that the volatile oil of *Elettaria cardamomum* seeds is mainly rich in phenolics and flavonoid compounds. Other constituents in the oil present in minor amounts are carbohydrates, protein, waxes and lipids. The major constituents of volatile oil of cardamom seeds are α-pinene, sabinene, β-myrcene, limonene, 1,8 cineole, γ-terpinene, terpinolene, linalool, linalyl acetate, terpinen-4-ol, α-terpineol, β-terpineol, α-terpinyl acetate, octyl acetate, nerly acetate, nerolidol, geraniol, geranial, β-caryophyllene, cis-trans farnesol and cis-cis farnesol. The sterol fraction was found to be β-sitostenone and γ -sitosterol. Phytol and traces of eugenyl acetate were also identified in cardamom seed oil.

Tijjani et al.[37] performed the phytochemical analysis of methanolic extract cardamom seeds . The extract concentrate yield was estimated to be 9.8%w/w which is brown in colour and oily in texture. Preliminary screening analysis results confirmed the presence of carbohydrates, tannins, cardioactive glycosides, terpenes, flavonoids, alkaloids and saponins. Anthraquinone was not found in the extract.

Husain et al.[38] isolated the volatile oil of the *E. cardamomum* seeds obtained from South India and reported that the oil was characterized by a large number monoterpenes (98%). These were present as hydrocarbons

(~10%), esters (~2%) and alcohols (~88%). The principal monoterpene was 1,8–cineole (85%).The remaining were *cis*-ocimene (3%) and alpha-terpinene (2%). Very low amount of sesquiterpenes like alpha-guaiene, caryophyllene oxide and nerolidol, were isolated from the volatile oil.

Bisht and his coworkers[39] revealed the presence of starch, proteins, lipids, terpinoids, flavonoids, glycosides, alkaloids and tannins by screening many extracts of *Elettaria cardamomum.* Terpinoid hydrocarbons namely lamonene, terpinenes, sabeinene and pinenes are recognized as predominant components. Roughly about 6% of the oil comprises of terpinols. Their findings suggest that the intense aroma of the spice is due to the presence of high cineole content. Gallic acid was the major phenolic compound present in the seed extract. Other glycosidic compounds isolated from the cardamom seed extract are subulin diglucoside, petunidin diglucoside and leucocyanadin glucopyranoside. Additionally, some constituents were seen in minor quantities namely pinenen, sabinene, cymene, terpinenol, nerolidol, terpinenes, bisabolene, protocatechualdehyde and protocatechuic acid. Both protocatechualdehyde and procatechuic acid are highly potent free radical scavengers.

Gochev et al.[40] separated volatile oil of cardamom seeds by Clevenger's apparatus and subjected it to Gas chromatography Mass Spectrometry (GC-MS) for the separation and identification of constituents present in the oil. Six compounds were separated at different retention time.

The mass fragmentation studies revealed the presence of 1,8- cineole and caryophyllene in the volatile oil.

Vavaiya et al.[41] subjected cardamom fruit powder to phytochemical screening after extraction with acetone and methanol. The phytochemical investigations were done by qualitative chemical tests, thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC) and spectrometry. Qualitative chemical examinations of methanol and acetone extracts showed presence of carbohydrates, flavonoids, amino acids, steroids, terpenoids, glycosides, tannins and phenolics. Total phenolic content in the Methanol and acetone extract were found to be 4.494 and 6.223 µg/5mg (0.09% and 0.124%w/w) respectively calculated in terms of Gallic acid. The phenolic compounds may contribute directly to the anti oxidative action. Phenolic compounds are effective hydrogen donors, which makes them good antioxidants.

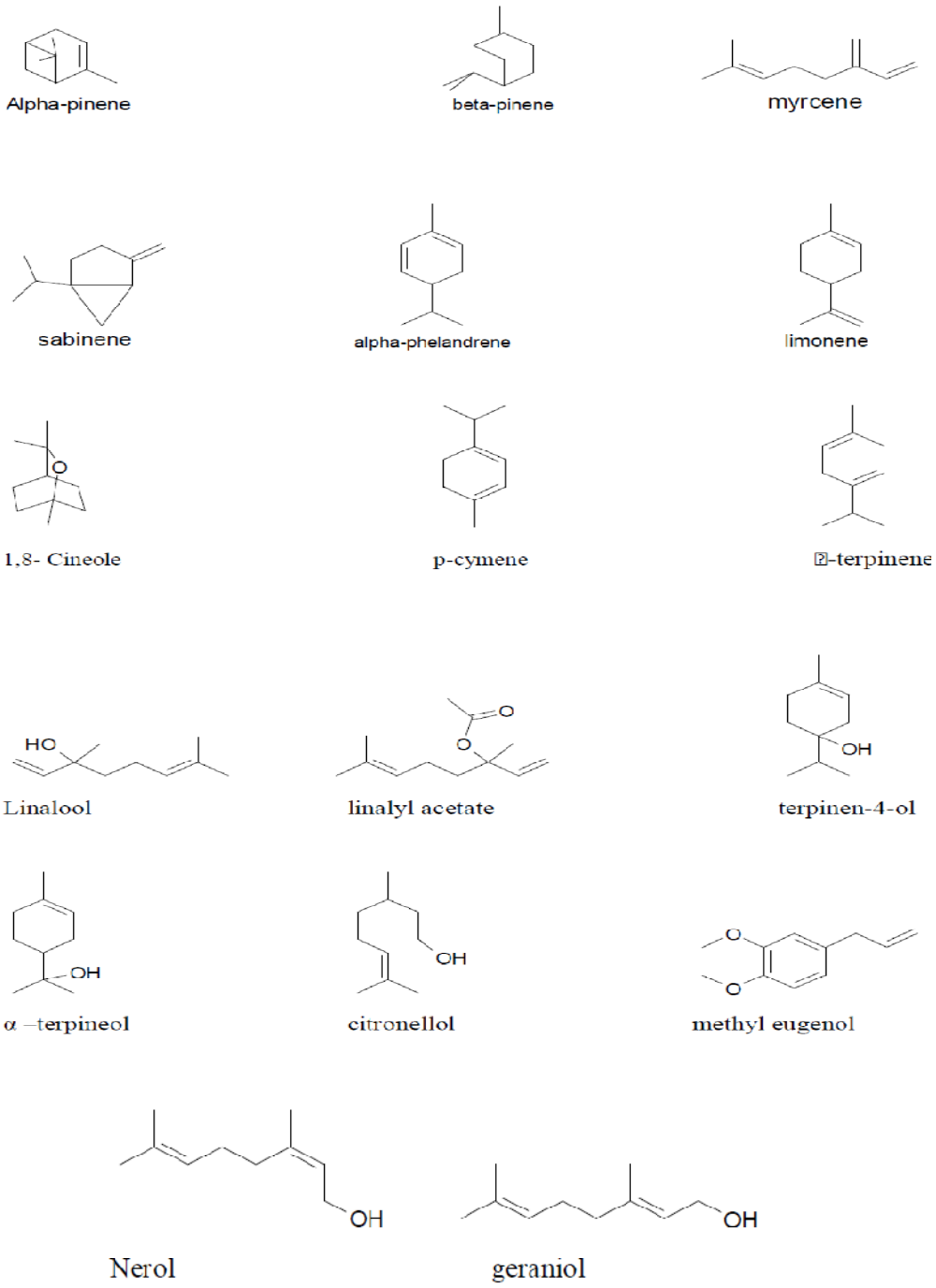

Fig 1: Chemical Structure of Major Constituents of *Elettaria cardamomum*[5]

[Adapted from Sharma et al. Therapeutic uses of Cardamom. Int J Drug Form Res 2011;2(6):102-8.]

4.1.6 Medicinal properties

Cardamom is an ancient spice and has the longest influence in India, its birthplace. The ancient Greeks and Romans also use it in food, medicines and perfumes. In various traditional systems of medicine like Matsya Purana, it is a constituent of an antivenom drug. In Ayurveda, the seeds are used as abortifacient, alexeritic, aromatic, sweet, cooling, carminative, cardiac tonic, digestive, diuretic, expectorant, appetite stimulant and found beneficial in asthma, bronchitis, strangury, haemorrhoids, renal and vesical calculi, halitosis, anorexia, dyspepsia, gastropathy and dyspepsia. In Siddha medicine, the dried fruit, seed and stem-bark are used to prepare drugs for various ailments. In the Unani system of medicine, cardamom containing preparations are used as antidote to poison, astringent, exhilarant and in nausea.[12,16]

The cardamom seeds have found to have antibacterial, gastroprotective, blood pressure lowering, anti-inflammatory, analgesic, antispasmodic, antioxidant, insecticidal, antiplatelet, sedative, anticonvulsant activities in various studies.[11-19,42]

4.1.7 Toxicity studies

Malti et al.[43] evaluated the toxicity of ethanolic extract of *Elettaria cardamomum* seeds on Swiss albino mice. The mice were divided into 4 groups were graded doses of the seed extract were given. The doses of ethanolic seed extract administered to the animals were Group-1 (0.003

mg/g), Group-2 (0.03 mg/g) and Group-3 (0.3 mg/g) and Group-4 (3mg/g) was administered The seed extract was administered daily orally to all animals for one week and were observed carefully for any signs of distress or death. During the 7-day follow up period, all the animals in Group 1 and Group 2 did not show any sign of stress or dyspnoea. Whereas, a statistical significant ($p<0.05$) decline in weight was observed in Group-3. 100% mortality was observed in all animals in Group-4 after 2 days of administration of extract.

Gilani et al.[15] performed acute toxicity test in mice. The test was using increasing doses of the crude extract of *Elettaria cardamomum*, given orally in 10 ml/kg volume to the test groups. Another group was administered saline (10 ml/kg) as negative control. The mice were allowed food ad libitum and kept under regular observation for 6 h while the lethality was recorded after 24 h. The lethal dose was found to be more than 10 g/kg.

4.1.8 Pharmacological screening done with *Elettaria cardamomum*

4.1.8.1 Antioxidant property

Amma KPAP et al.[44] studied the antioxidant activities of *Elettaria cardamomum* seeds using dichloromethane, ethyl acetate, methanol and water extracts. The antioxidant activity of different extractives were measured in terms of hydrogen donating or radical scavenging ability using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. It was found that DPPH radical scavenging activity of all extracts

increase with increase in concentration. The highest antioxidant activity observed with ethyl acetate extract. The increase in activity could be due to the enrichment of phenolic compounds in cardamom seeds.

Prakash et al.[45] tested the in-vitro antioxidant activity using the DPPH method. The study was done using four groups; Group-I (Standard-Ascorbic acid), Group-II (Aqueous extract of cardamom seeds) and Group-III (Ethanolic extract of cardamom seeds). The antioxidant activity was assessed by using the concentration of drug leading to 50% reduction in free radicals (IC50 value). The mean IC50 values of Group-I, Group II and Group III are 2 µg/mL, 22 µg/mL, 8 µg/mL respectively. The study concluded that the *in-vitro* antioxidant potential of *Elettaria cardamomum* ethanolic extract of seeds was proved better than its own aqueous extract but was inferior to ascorbic acid.

Ashadevi et al.[18] determined the antioxidant potential of *Elettaria cardamomum* methanolic seed extract using two methods namely, DPPH method for free radical scavenging activity and Riboflavin-Nitroblue tetrazolium (NBT) method for superoxide anion scavenging activity. *E.cardamomum* extract showed striking superoxide anion scavenging activity and the DPPH free radical scavenging percentage was 11.77%.

Gochev et al.[40] evaluated the *in vitro* antioxidant activity of tetrafluroethane extract of cardamom seeds. The radical scavenging capacity was determined according to the DPPH method. The cardamom

extract showed 55.2% inhibition of DPPH radical at a concentration of 100 mg/ml and the IC50 value was 63.3 mg/ml. In comparison with strong antioxidants such as ascorbic acid and rutin, which are traditionally used in cosmetics and food industry, the cardamom extract possesses considerably lower antioxidant activity.

Goyal et al.[46] demonstrated the antioxidant activities of cardamom in albino rats. All the animals were randomized into three groups. Animals in each group were administered orally either sterile water, 100 mg/kg or 200 mg/kg of the cardamom seed extract for one month. After one month of drug administration, cardiac puncture was done and the collected blood was estimated for reduced glutathione (GSH) content and malondialdehyde (MDA) levels. An attenuated reduced GSH content and accentuated MDA levels indicate lipid peroxidation. Additionally, the cardamom pretreated groups showed higher levels of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase than the control group. All these evidences suggests the free radical scavenging and antioxidant potential of *Elettaria cardamomum.*

4.1.8.2 Anti-inflammatory and analgesic property

Mehjabeen et al.[47] analyzed the anti-inflammatory activity of the crude extract of *Elettaria cardamomum* seeds using formalin and carrageenan tests in Wistar albino rats. The crude extracts of cardamom seeds in three increasing doses 200, 300 and 500 mg/kg were given orally to the animals. It was observed that *E.cardamomum* produced dose

dependant inhibition of formalin induced paw licking and carrageenan induced paw edema. a significant percentage of inhibition. The percentage of inhibition of carrageenan induced paw edema were (25.1%; 36.7%; 40.7%, 30.3%; 34.3%; 44.3%, 22.39%; 29.5%; 44.7% respectively at 200, 300 and 500mg/kg oral dose) compared to standard drug aspirin. The percentage inhibition of formalin induced paw licking was significant in the dose of 500 mg/kg orally and found to be 84.61% when compared to aspirin (standard).

Kumar et al.[16] studied the analgesic activity of the oil extracted from *Elettaria cardamomum* seeds using chemically induced writhing model in Swiss albino mice. The animals were administered 0.02% solution of p-benzoquinone intraperitoneally to induce writhing. It was observed that 50% of the animals pretreated with a dose of 233 µL/kg of the oil were protected against writhing.

4.1.8.3 Sedative and Anticonvulsant property

Gilani et al.[15] investigated on Swiss albino mice, the sedative properties of cardamom seeds. The mice were divided into five groups with five in each group. Group-A received 10 ml/kg of 0.9% normal saline i.p. and Group-B received diazepam at dose of 5 mg/kg i.p. Rest of the animals were administered orally *Elettaria cardamomum* crude extract (Ec.Cr) of seeds in graded doses. After 30 minutes of i.p administration and one hour of oral administration all the animals were given pentobarbitone at a dose of 75 mg/kg i.p. The pentobarbitone-induced

sleeping time was recorded in all animals. The mean sleeping time in the control group was 117 minutes while with the standard drug treated group was 348 minutes, which was statistically significant ($p<0.05$). Ec.Cr (30–300 mg/kg) prolonged the pentobarbital-induced sleeping time in mice. The mean sleeping time was prolonged in a dose dependant manner in the test groups where Ec.Cr was administered in graded doses. Maximum prolongation was seen with Ec.Cr at a dose of 300 mg/kg. The mean sleeping time for 300 mg/kg Ec.Cr treated group was 277 minutes, which was statistically significant to the control group. Moreover, there was no significant difference in the mean sleeping time between the diazepam pretreated group and 300 mg/kg Ec.Cr pretreated group. Further, the study was concluded by mentioning that the prolongation of sleeping time may contribute to the rationale of using cardamom in seizure disorders but more evidences are needed to confirm whether it has any direct anticonvulsant effect.

Achilya et al.[48] evaluated the neuropharmacological activities of 'Unmadnashak Ghrita' (UG). UG is an ayurvedic formulation containing *Ferula narthex* (6 g), *Gardenia gummifera* (6 g), *Elettaria cardamomum* (6 g), *Bacopa monneri* (6 g), and cow's ghee (clarified butter fat) (76 g).. The formulation showed CNS-depressant activity in gross behavioural test, potentiated pentobarbitone sleeping time and there was significant decrease in spontaneous locomotor count in mice. The formulation also protected mice from maximal electroshock (MES) and pentelenetetrazole (PTZ)

induced convulsions. These results suggest that UG has CNS-depressant and anticonvulsant activity in mice.

4.1.8.4 Gastroprotective property

Jamal et al.[14] evaluated the ability of crude methanolic extract, essential oil , petroleum ether soluble and insoluble fractions of methanolic extract, to inhibit the gastric lesions induced by aspirin, ethanol and pylorous ligature in rats at doses of 100–500, 12.5–50, 12.5–150 and 450 mg/kg. In addition their effects on wall mucus and gastric acid output were recorded. It was found that all fractions reduced the number of gastric lesions induced by ethanol and aspirin but not those induced by pylorus ligation. Crude methanolic extract proved to be active reducing lesions by about 70% in the ethanol-induced ulcer model at 500 mg/kg. The petroleum ether fraction reduced the lesions by 50% at 50 and 100 mg/kg. In the aspirin-induced gastric ulcer, the best gastroprotective effect was found in the petroleum-ether fraction, which inhibited lesions by nearly 100% at 12.5 mg/kg. The petroleum ether extract at doses ≥12.5 mg/kg proved to be more active than ranitidine at 50 mg/kg.

Wandale and his co-workers [49] analyzed the gastroprotective property of a polyherbal formulation containing licorice, ginger and cardamom by using NSAID induced ulcer model and pylorus ligation model. The gastroprotective activity was evaluated using histopathological examination. In the NSAID-induced ulcer model, percentage of gastroprotection in rats pretreated with the formulation at a dose of 100

mg/kg, 250 mg/kg and 500 mg/kg was observed to be 26% 59% and 79% respectively. In the pylorus ligation model, the percentage of gastroprotection in animal pre-administered with the formulation at graded doses were 58%, 68% and 87% respectively.

4.1.8.5 Blood pressure lowering property

Gilani et al.[15] evaluated the blood pressure (B.P) lowering effect of crude extract of *Elettaria cardamomum* (Ec.Cr) on anaesthetized rats. The rats were pretreated with four ascending doses of 3, 10, 30 and 100 mg/kg of the extract. A dose-dependent decline in blood pressure was observed in rats. The animals showed a fall in B.P of approximately 7%, 16%, 37% and 53%. Moreover, an incomplete blockade of this B.P lowering activity was demonstrated using an anticholinergic agent, atropine at a dose of 1mg/kg. The diuretic activity in these animals were also seen. The volume of urine output of rats/ 100g BW/ 6 hours in control (0.9% saline) group and standard (furosemide) group were 3mL and 7mL respectively. The rats pretreated with the crude extract of cardamom showed diuretic activity at 3 and 10 mg/kg with a volume of 5 and 5.5 mL of urine respectively.

Verma et al.[19] studied the antihypertensive potential of *Elettaria cardamomum* fruit powder in individuals with Stage I hypertension. Twenty, newly diagnosed individuals with primary hypertension of stage I were administered 3 g of cardamom powder in two divided doses for 12 weeks. Blood pressure was recorded initially and at 4

weeks interval for 3 months. Cardamom administration significantly reduced the blood pressure at the end of first end. At the end of 3 months blood pressure reached within the normal range as defined by Joint National Committee (JNC) - VII report.

4.1.8.6. Antimicrobial property

Kaushik et al.[13] studied the anti-bacterial potential of dry fruit extract of *Elettaria cardamomum* by the standard agar well diffusion assay. Extracts of organic solvents like ethanol, methanol, ethyl acetate and hexane were tested. The antibacterial activity of the each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced at the end of incubation period. A total of six bacterial strains including both Gram-negative and Gram-positive bacteria (*Escherichia coli*, *Salmonella typhi*, *Bacillus cereus*, *Bacillus subtilis*, *Streptococcus pyogenes*, and *Staphylococcus aureus* were selected to assess susceptibility patterns against the extracts. Aqueous extract was found inhibitory for all the test bacteria and *S. aureus* was found the most susceptible bacterium to the extract. Ethyl acetate extract was active against all microflora except *S. typhi*. Ethanol extract was found inhibitory against all the test bacteria but the activity was found lower than previously discussed two extracts. Methanol extract was active only against *S. aureus* and *E. coli*. Hexane extract was found completely inactive against all the test organisms. Inhibition range for *S. typhi* and *S. pyogenes* was observed very mild against ethanol and aqueous extracts.

Husain et al.[38] examined antibacterial activity of cardamom oil against E. coli, S. aureus and B. subtilis and antifungal activity against Aspergillus niger, A. fumigatus, A. flavus and Candida albicans. The volatile oil showed significant antimicrobial and antifungal activity in comparison to standard, Tetracycline and Fluconazole.

4.2 Epilepsy

Epilepsies are a group of chronic neurological disorders characterized by brief, recurrent, paroxysmal discharges of electrical activity from the different locations of the brain. The discharge of electrical activity is evidenced by repetitive firing of action potentials evoked by sustained depolarization. Each episode of neuronal dysfunction is called a seizure. Seizure symptoms vary according to the location of seizure activity and may include prominent motor symptoms (convulsions), loss of consciousness, alternations in sensory, cognition and emotions. Convulsions are violent involuntary spasmodic contractions of the skeletal musculature.[50] Depending upon the area or location in the brain involved and the nature of convulsions produced, seizures can be classified into different types.

4.2.1 Classification of Seizures

The International League Against Epilepsy commission has proposed a classification of seizures based on clinical features and EEG findings as follows :-

4.2.1.1. Generalized seizures

Generalized seizures affect both cerebral hemispheres from the beginning of the seizure. They produce loss of consciousness, either briefly or for a longer period of time. They are sub classified into several types like generalized tonic-clonic seizures (GTCS), tonic, myoclonic, atonic and absence seizures. [51-53]

4.2.1.1.A. Tonic-Clonic seizures

Generalized tonic-clonic seizures (GTCS) are also called as grandmal epilepsy. These seizures are characterized by different types of aura, followed by a sudden loss of consciousness, tonic contraction of skeletal muscles, inability to maintain posture and a typical 'cry' produced due to the violent contraction of respiratory muscles resulting in forced expiration of air. Moreover, the muscles become rigid and the individual falls to the ground in an opisthotonic position, often sustaining injury. There may be a brief cessation of breathing. This phase is followed by a state of muscle relaxation. The loss of consciousness may be for a few minutes or longer. Then consciousness is regained and the individual becomes dizzy and disoriented. During the seizure, there may be urinary or fecal incontinence. During the postictal period, the person may experience headache, drowsiness and extreme weakness. The EEG shows low-voltage fast frequency [10Hz] wave pattern during convulsive period.[51]

4.2.1.1.B. Tonic seizures

An aura is followed by convulsions associated with a tonic phase characterized by contraction of muscles. Epileptic cry due to contraction of the laryngeal muscles may be produced. There is no clonic phase as seen in generalized tonic-clonic seizures. Then patient goes into a phase of coma which may last for about half an hour.[51-53]

4.2.1.1.C. Atonic seizures

Atonic seizures or drop attacks are characterized by brief period of impairment of conscious and unable to maintain an erect posture. There is no associated tonic muscular contractions. The patient simply drops to the ground without any apparent cause. They occur in children accompanied by other forms of seizures. [51-53]

4.2.1.1.D. Myoclonic seizures

These type of seizures are characterized by sudden, quick, single or repetitive skeletal muscle contractions involving a part or the whole body. When entire body is affected, there is violent fall without loss of consciousness. They are often idiopathic or may occur as a major neurological symptom in conditions like uremia or hepatic failure. [51-53]

4.2.1.1.E. Absence seizures

Also called petitmal epilepsy. It is characterized by sudden loss of consciousness without convulsive muscular activity or loss of postural control. They are very brief and may be unnoticed, lasting a few seconds to a few minutes. The sudden loss of consciousness may be associated with minor motor changes such as eyelid fluttering, small chewing movements of the mouth and mild shaking of the hands. At the end of the absence seizures, the patient usually regains consciousness quickly. They occur in young children of 6-14 years of age and the episodes occur several times in a day. The EEG shows a characteristic 3Hz spike and wave discharge during the attack and normal activity in the interictal period. [51-53]

4.2.1.2. Partial seizures

Partial seizures or Focal seizures originate in a localized area of the brain, most commonly the medial temporal lobe or inferior frontal lobe. There is no loss of consciousness in partial seizures. The convulsions are restricted to a group of muscles (Jacksonian motor seizures) or confined sensory disturbance (Jacksonian sensory seizures) or associated with cognition (psychomotor seizures) depending on the area of cortex involved in the seizure activity. Some special features of focal motor seizures include atypical muscle movements which may be initially restricted to smaller areas like the fingers and eventually

progress to include a major portion of the extremity and become generalized. This phenomenon is known as "Jacksonian march," representing the spread of seizure activity over a larger region of motor cortex progressively. The patients can also experience a postictal neurological deficit (Todd's paralysis) for minutes to many hours. *Epilepsia partialis continua* is a very rare situation where the seizure activity can continue for hours or even days. [51-53]

4.2.1.2.A. Simple Partial Seizures

Simple partial seizures are also called as cortical focal seizures. Here, consciousness is intact and convulsions are localized to a group of muscles or shows sensory disturbances depending upon the area of cortex involved. They can occur with motor, sensory, autonomic or psychic symptoms. Simple partial seizures are manifested usually in the form repeated contractions of muscles in one part of the body without any impairment in consciousness. Each muscular contraction is caused by the discharge of neurons in the corresponding area of the contra lateral motor cortex. The muscular activity may be confined to one area or may spread from the affected area to involve contiguous ipsilateral body parts (for example, right thumb to right hand or right side of face). This is also referred to as Jacksonian epilepsy. The EEG shows regularly occurring spiking in the affected area of brain. Simple partial seizures may be accompanied by sensory symptoms like paraesthesia. [51-53]

4.2.1.2.B. Complex Partial Seizures

Usually complex partial seizures affects the temporal lobe of the cortex and cause behavioral disturbances, hence, they are referred to as, "temporal lobe epilepsy" or "psychomotor epilepsy". The attacks are peculiar with uncertain behavior and undetermined movements, emotional changes lasting for 1-2 mins and associated with impairment of consciousness. An aura often precedes the attack. The seizure focus in located on the temporal lobe. During complex partial seizures, there may be cessation of normal activity with the presence of only minor motor activity such as lip smacking, swallowing, walking aimlessly or picking at one's own clothes. These peculiar behavior are called automatisms. The EEG shows unilateral and bilateral spikes or slow wave discharge over temporal or frontotemporal regions both during seizures and in between. [51-53]

4.2.1.2.C. Secondarily Generalized

Both simple and complex focal seizures can become generalized. The seizure activity begins at a focus and manifests as a partial seizure but soon evolves into GTCS with loss of consciousness.[51-53]

4.2.1.3. Unclassifiable seizures

Some seizures do not come under generalized or focal seizures and hence categorized as unclassifiable seizures. Unclassifiable seizures include epileptic spasms, neonatal seizures and febrile convulsions. [51]

4.2.1.3.A. Neonatal seizures

It is seen usually around the first week of birth and is caused by deficiency of vitamin B_4 due to reduced GABA synthesis. These movements are mostly associated with EEG changes. They often involve one extremity or one side of the body. The rhythm can be of clonic movements, tonic movements or myoclonic seizures and is usually slow at 1-3 movements per second. Focal and multifocal myoclonic seizures typically are not associated with EEG changes. [51]

4.2.1.3.B. Hypsarrythmia

Also called as infantile spasms. The proximal muscle groups are in a state of continuous flexion or extension. EEG shows diffuse giant slow waves with multifocal spikes and sharp waves. EMG has characteristic rhomboid pattern. A toddler who has centrotemporal spikes (rolandic epilepsy) has a good prognostic profile and does not require a prolonged course of antiepileptic drugs. Clinical classification of such a seizure may be difficult as many different seizures has similar characteristics when they manifest in a complex way. For example, the

clinical manifestation of a child with absence seizures appears almost similar to those of another patient presenting with complex partial epilepsy. An adjunct to the classification of epilepsy using an EEG is helpful because of the variations in seizure manifestation in this age group. [51]

4.2.1.3.C. Febrile convulsions

Febrile convulsions are seen in children between the age group of 9 months to 5 years. These convulsions occur when the child has high grade fever ($\geq 39^0$C), usually on the first day. Its causative factor is not known. Child presents with an epileptic episode usually on first or second day of fever lasting for about 2-3 minutes which resolves rapidly with a post ictal phase of drowsiness. Laboratory investigations include lumbar puncture, serum electrolytes, plasma glucose and toxicological screening. In order to prevent such episodes, the child is given tepid sponging and prophylactic paracetamol to reduce the body temperature and also prophylaxis with diazepam at the onset fever itself.[51]

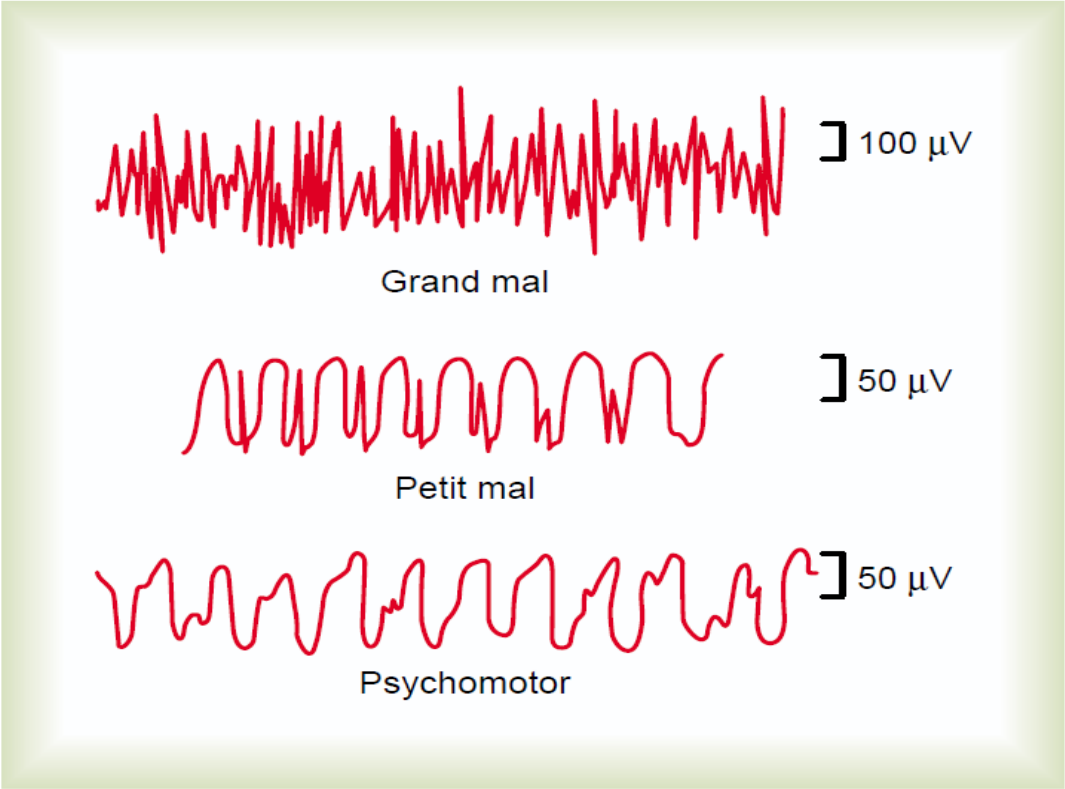

Fig 2: Electroencephalogram in different types of epilepsy [53]

[Adapted from Hall JE. Guyton and Hall Textbook of Medical Physiology.12th ed.Philadelphia:Saunders Elsevier 2013.p.820]

4.2.2 Etiology

Neuronal hyperexcitability is the ultimate cause of seizure activity within the brain. There are multiple factors that can alter the normal homeostasis and turmoil the neuronal activity at cellular or biochemical levels. Seizures are triggered by the hyperexcitable and unstable state of neurons that may occur due to several medical conditions from genetic mutations to traumatic injury to brain. A genetic predisposition has been distinguished in many forms of primary generalized epilepsy. The likelihood of seizures and epilepsy is accentuated in patients with head injury, stroke,

mental retardation or cerebral palsy. Moreover, the occurrence of seizure activity is directly proportional to the intelligence quotient (IQ), which is a measurement of degree of mental retardation. Neurodegenerative disorders like Alzheimer's or Parkinson's disease in the elderly are also associated with triggering of seizure activity within the brain. Seizures can also occur without any established etiology (idiopathic). [50]

Apart from organic diseases of the brain, many physiological conditions and drugs can induce seizure in susceptible individuals. Physiological conditions that can predispose to epilepsy are hyperventilation, excessive sleep, sleep deprivation, emotional stress, puberty, menstruation (catamenial epilepsy) and pregnancy. Many drugs like alcohol, theophylline, anti-psychotics like chlorpromazine, clozapine and anti-depressants like clomipramine, bupropion can decrease the seizure threshold.[51]

4.2.3 Pathophysiology

Seizures can occur either due to excessive excitation or disrupted inhibition of neurons in the brain. These abnormal electrical events can be recorded in the EEG as spikes with different frequency. At the beginning of an event, a small population of neurons will undergo aberrant excitation and fire abnormally. Gradually, this excitation spreads either focally to produce partial (focal) seizures or more extensively to involve other adjoining areas to produce generalized seizures. The clinical features depends upon the area involved along with degree and intensity of the excitation. [50]

The aberrant hyper-excitation of neurons is probably due to the following mechanisms:- (1) up/down regulation of the neuronal membrane ion channels and/or receptors; (2) modulation of the second messengers; (3) alternations in the uptake and metabolism of neurotransmitters; (4) attenuation of physiological inhibitory circuits and synapses. Transient disparity between excitatory (glutamate) and inhibitory (GABA) neurotransmitters may also trigger seizures in vulnerable individuals. [50,51]

4.2.4 Animal Models of Epilepsy

The preclinical studies using animal models have played a very important part in the discovery of various clinically effective antiepileptics. The currently used models represent epileptic seizures rather than epilepsy as such.[54]

4.2.4.1 Electrically induced seizure models

Drug screening methods using these models are effective against GTCS and focal seizures. Using these methods, the potential anticonvulsant activity of a drug can be determined, but it do not give any idea regarding the mechanism of action of drug.[55]

4.2.4.1.A Maximal Electroshock induced Seizure (MES) model

This model was introduced in the year 1938 by Merritt and Putnam. The animals of choice for this model are rats and mice. The corneal and ear electrodes are used to apply electrical stimulation from a stimulator that supplies a steady current of 50 mA (mice) or 150 mA

(rats) at a frequency of 50-60 Hz for 0.2 secs duration. A steady voltage of 250 V (mice) or 750 V (rats) is used. After application of the electrical stimulus, all animals are observed for a period of 2 minutes. The resultant seizure may progress through different phases like phase of tonic limb flexion (1.5 secs) followed by the phase of tonic limb extension (10 secs) and eventually followed by a variable short clonic interval. Death may occur due to asphyxia. An antiepileptic drug is considered as efficacious if it is able to suppress the phase of tonic hind limb extension.[54-56]

4.2.4.1.B Threshold models

These models are used to ascertain the capacity of a drug to regulate the seizure threshold for tonic hind limb extension. When used in parallel to MES test, this model is useful to screen drugs which are effective against GTCS. These drugs usually accentuate the seizure threshold in grandmal seizures. Mice or rats are the most commonly used animals for the experiments.Electrical stimulation is given by applying corneal or ear electrodes which delivers a steady current or voltage at a frequency of 50-60 Hz for 0.2 seconds duration. Threshold is determined as the current or voltage which induces hind limb extension in 50% of the animals. Elevation of seizure threshold is taken as the measure of efficacy of the test drug. [54-56]

4.2.4.1.C Kindling models

The kindling phenomenon is observed after repeated administration of submaximal electrical or chemical stimulus resulting in progressive intensification of the stimulus-induced seizure activity and terminating in a generalized seizure. Kindling demonstrates the fact that 'epilepsy induces epilepsy'. Adult Sprague-Dawley rats are used for this experiment and amygdala is the most commonly chosen region for kindling. [54-56]

Electrical stimulation is done through an electrode placed in the right or left amygdala. After a recovery period of 1-2 weeks, daily electrical stimulus (500 μA, 1 millisecond monophasic wave pulses for one second at a frequency of 50-60 Hz) is applied through the electrode. Daily electrical stimulation results in seizures, which evolve through five stages:

- Stage 1 – Reduced movements, eyes may close, repetitive sniffing, quivering of vibriasse
- Stage 2 – Clonic facial spasms and head nodding
- Stage 3 - Stage 2 + Clonic forelimb spasms
- Stage 4 – Rearing associated with by bilateral forelimb clonus
- Stage 5 – Stage 4 + loss of balance and falling associated with generalized clonic seizures - [Fully kindled state] [54-56]

If the electrical stimulation is prolonged for a couple of weeks, the rats develop unprompted epileptic seizures. Four different measures for drug latency (seizure latency, seizure severity, seizure duration and after discharge duration) are recorded. [54-56]

4.2.4.2 Chemically induced seizure models

4.2.4.2.A Pentylenetetrazole (PTZ) test

PTZ is chemically a tetrazole group containing compound having a uniform effect in a large number of animal species. Its action is through inhibition of GABA neurotransmission. It is a useful and commonly used model for screening drugs effective against petitmal epilepsy or absence seizures. The seizure activity can be suppressed by drugs like ethosuximide and valproate. Rats or mice are used. The dose of PTZ is 70 mg/Kg in rats and 90 mg/Kg in mice. All animals in control group develop seizures within 30 minutes in a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal clonic seizures and death. The first episode of clonic jerking lasting for 5 sec or the first episode of clonic seizure with loss of righting reflex is taken as the end point. [54-56]

4.2.4.2.B Other Chemoconvulsants

Bicuculline, picrotoxin, penicillin, isoniazid, thiosemicarbazide, allylglycine, strychnine, pilocarpine, N-methyl D aspartate, kainic acid, gamma hydroxybutyric acid and quinolinic acid. [54]

4.2.4.3 Focal lesions induced seizure models

Seizures are induced with cortically implanted metals like alumina cream / gel, cobalt, tungstic acid or injection of iron into the brain cortex. Topical aluminium hydroxide gel model is most commonly used. Miscellaneous chemicals which are used to produce focal lesions are - intrahippocampal injections of kainic acid, topical applications of penicillin, cholinergics, picrotoxin, bicuculline, strychnine, zinc etc. [56]

4.3 Pain

Pain is a subjective experience which cannot be clearly defined or quantified satisfactorily. The International Association for the study of pain has defined it as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Physiologists describe nociception as signals reaching the central nervous system resulting from activation of specialized sensory receptors called nociceptors that provide information regarding the tissue damage where as pain refers to the unpleasant emotional experience that usually accompanies nociception. There are classically two components in the pain perception: (a) The nociceptive component - disabling ,unpleasant sensation evoked by the noxious stimuli and (b) The affective component - the psychological response associated with the pain.[57]

4.3.1 Types of pain

4.3.1.1 Acute and chronic pain

Acute pain usually follows traumatic tissue injuries and is limited to less than one month of duration. The onset is sudden and resolves with healing. It is usually accompanied with autonomic symptoms like tachycardia, tachypnoea, sweating etc. Acute pain is usually considered to as a 'good pain' as it serves to be a protective mechanism. The withdrawal reflex is a classical example of this protective mechanism of pain. Chronic pain is considered as a bad pain as it persists even after recovery from the injury. Chronic pain results from nerve injury, toxin associated nerve damage, ischemia etc and this type of pain is usually refractory to the analgesic drugs.[57-58]

4.3.1.2 Fast and slow pain

Fast pain is usually felt within 0.1 second after a pain stimulus is applied, whereas slow pain is felt only after 1 second or more and then increases slowly. It is also described as sharp/pricking/acute/electric pain. Typical examples of fast pain includes the pain felt after needle prick or burns. Fast pain is very superficial and deeper tissues are not involved.

Slow pain is also referred to as slow burning pain, aching pain, throbbing pain and chronic pain. This type of pain is usually

associated with tissue damage. It can affect superficially as well as any deeper tissues. [57-58]

4.3.1.3 Nociceptive and neuropathic pain

Nociceptive pain arises due to direct stimulation of the peripheral nerve endings.eg: pain associated with wounds,burns,angina etc and Neuropathic pain due to injury of nerve fibres resulting in the dysfunction of the pain perception system in the peripheral and the central nervous system. It can occur following acute events (amputation, spinal cord injury) or systemic diseases (diabetes, cancer). It is excruciating and difficult to treat also. [57-58]

4.3.1.4. Integumental and visceral pain

Integumental or somatic pain is peripheral, localized, aching in nature where as visceral pain is poorly localized and associated with nausea and autonomic disturbances and is often referred to the cutaneous areas. [57-58]

4.3.2 Pathophysiology of Pain

The pathophysiology of pain involves a complex array of neural networks in the brain that are acted on by afferent stimuli to produce the experience know as pain. It can be physiologic and protective (nociceptive) or pathophysiologic and harmful (e.g. neuropathic). [58]

Nociceptive pain can be considered protective and physiologic. The first step leading to the sensation of pain is stimulation of nerve endings known as nociceptors. These receptors are found in both somatic and visceral structures. When mechanical, thermal and chemical stimuli sensitize these receptors, they may lead to activation and release of substances like bradykinins, nerve growth factor, prostaglandins, histamine, interleukins, tumour necrosis factors, serotonin, substance P and others. A-δ and C fibres are the afferent nociceptive pain fibres. These fibres synapse in the dorsal horn of spinal cord where they release a large number of excitatory neurotransmitters like glutamate, substance P and aspartate. These neurotransmitters activates the ascending spinothalamic tract. The thalamus acts as a relay station as these pathways ascend and pass the impulses to central structures where pain can be processed. The body modulates pain through a number of neurotransmitters acting on different receptors like the endogenous opioids (enkephalins, dynorphins, β-endorphins), endogenous peptides (Neuropeptide Y). Descending pathway of pain also controls pain transmission. The neurotransmitters which play a major role in modulation of pain in the descending pathway are γ-aminobutyric acid (GABA), endorphins, enkephalins, serotonin and noradrenaline. [57-58]

Pathophysiologic pain is distinctly different from nociceptive pain. This type of pain usually does not involve a noxious stimulus but is attributed to the deranged functioning of the nervous

system. All chronic pain like neuralgias (postherpetic/trigeminal), diabetic neuropathy, fibromyalgia etc fall into this category. Damage to nerves or certain diseased states can alter the functioning of both peripheral and central neurons leading to gradual accentuation in the release of neurotransmitters from dorsal horn neurons. [57-58]

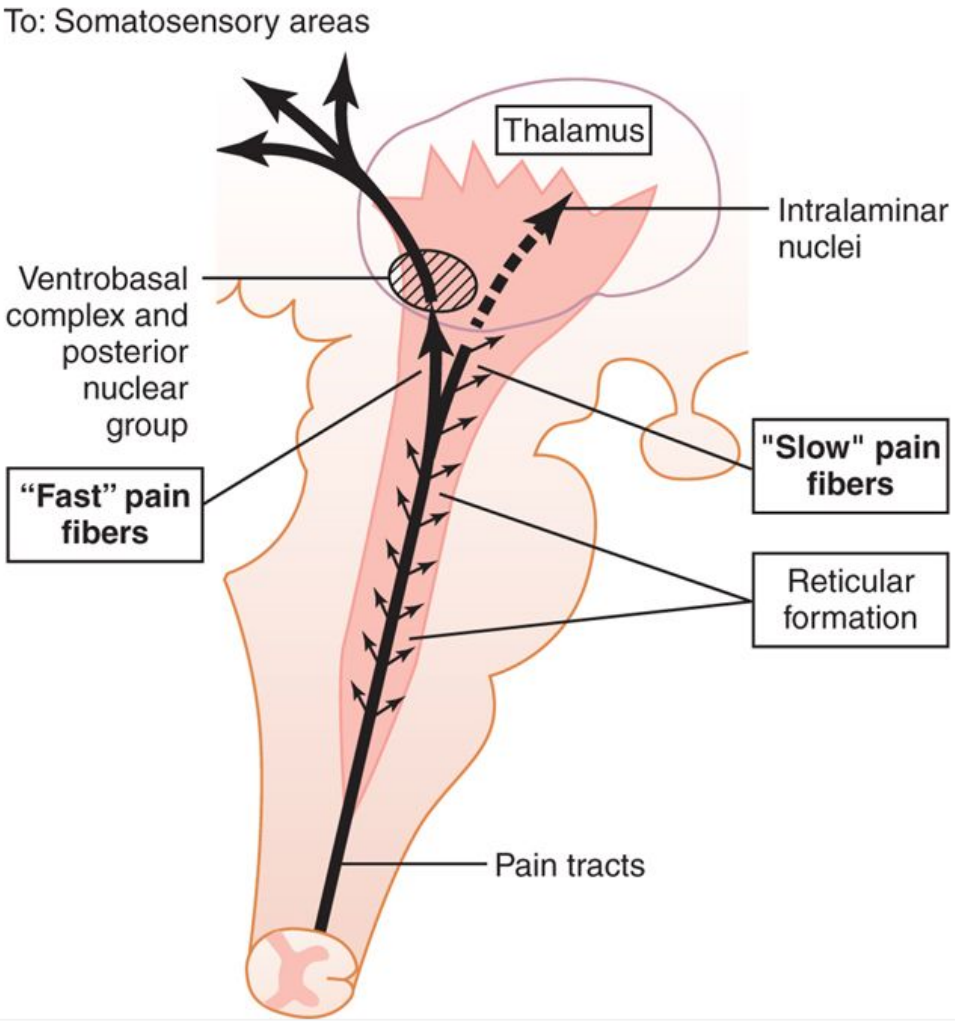

Fig 3 : Transmission of pain signals into the brain stem, thalamus, and cerebral cortex by way of the fast pain and the slow pain pathway. [57]

[Adapted from Hall E. Guyton and Hall Textbook of Medical Physiology.12th ed.Philadelphia:Saunders Elsevier 2013.p.700]

4.3.4 Animal models of pain

Pain models or Analgesic models are explored mainly in rodents to indicate various types of pain and aimed at :-

- Studying pain transmission
- Identifying new targets in pain modulation
- Discovery of novel compounds in pain management.

Since, lack of verbal communication in animals is doubtlessly a major hurdle in the evaluation of pain, the physical signs elicited are regarded as the most reliable signals of pain sensation. The signs include motor, autonomic and behavioural responses to pain :-

- Motor:- Withdrawal, Jumping, Contractures and Vocalization
- Autonomic:- Mydriasis, Hyperpnoea, Tachycardia and Hypertension
- Behavioural:- Reactions like escape, avoidance and hostile. Modifications of social, food, sexual and sleep habits. [53-55]

Analgesic screening models commonly used look predominantly on evoked pain (experimental pain under controlled conditions) which may significantly vary from the actual clinical pain as seen in humans.[60]

4.3.3.1 Models using thermal stimulus

Thermal stimulus is applied on the skin and theses tests do not involve other deep tissues. Usually, these tests rely on an escape behavior/withdrawal reflex or vocalization as an index of pain. The animal typically reacts by withdrawing itself immediately away from the stimulus.

These models are used to differentiate between the centrally acting opiate and non opiate analgesics. These models are used to assess the threshold for response to a high intensity acute painful stimulus. False positive results can occur with sedatives by prolonging the reaction time.[60-62]

4.3.3.1.A Hot plate method

Most commonly used model to evaluate centrally acting analgesics. Acute pain is induced using thermal stimulus. Rodent paws are not only highly sensitive to heat but also not damaged easily, hence, preferred for pain evaluation. Rodents are placed on electrically heated surface of a hot plate. The apparatus have an inbuilt thermostat knob to maintain the temperature of 55 ^{0}C. The animals quickly respond by jumping, licking or withdrawal of paws. The time between onset of stimulus and response of the animal (latency period) is recorded using stopwatch and is compared between the control, standard and test groups. The cut off time for observation is 15 to 20 seconds for mice and 20 to 30 seconds for rats. [60-62]

4.3.3.1.B Tail-Flick test

The principle of this test is that heat is used as noxious stimulus and the animal responds by flicking away its tail. The advantage of this model is that there is minimal inter animal variation. This test is widely employed in screening of centrally acting analgesics. Two forms of the tail-flick test are frequently employed: *(a) Radiant heat method:-*

Application of heat on to a small spot of tail *(b) Tail Immersion method:-* Immersion of part of tail in water at a preset temperature. [60-62]

4.3.3.1.B.a Radiant heat method

The application of thermal radiation to a specific spot on the tail of the rodent will result in tail withdrawal reflex reaction. This reaction time is recorded and is often referred to as "tail-flick latency". The radiant heat is applied using an electric equipment called "tail-flick analgesiometer". The intensity of the current flowing through the filament can be controlled by using a rheostat which is incorporated to the meter. Using a stop watch or timer, the tail withdrawal response time is recorded. The main advantage of this method is that it has least inter-animal variability. The cut off time is usually 15 seconds to prevent the skin from burning. [60-62]

4.3.3.1.B.b Tail immersion method

This method was based on the observation that centrally acting analgesics prolong the tail withdrawal reflex induced by immersing the distal part of the tail in warm water. The animals are trained to acclimatize and then they are placed in separate cages with their tail hanging freely. The distal 5 cm is marked and is immersed in a beaker filled with warm water at 55°C. The cut off time is 15 seconds to prevent any damage to the tail skin. Reaction time (tail withdrawal reflex) is observed with a stop watch. Normal time for response is 1-5

seconds. Centrally acting analgesics like morphine can prolong the reaction time of the tail-withdrawal reflex in rats. [60-62]

4.3.3.2 Models using electrical stimulus

These models are non-invasive and the results produced are quantifiable, reproducible and well synchronized afferent signals are being produced. But the limitation is that these electrical stimulus are non-natural as confronted by the animals in their normal habitat. Such stimuli excite all peripheral sensory fibres, which are not directly involved in pain. Thus, studies on peripheral transduction mechanisms are difficult with electrical stimulus. Variations can occur due to the differences in the impedances of tissues being stimulated. [60-62]

4.3.3.2.A Grid shock test

This method was first described in mice by Blake et al. Rats or mice are commonly used. The animals are kept in plastic boxes with steel wires spaced about 1 mm apart are tightened to the floor of the box. Electric stimulus is applied at a frequency of 30Hz for 2 milliseconds duration. The output of the stimulator is connected to the wires in an alternate fashion. A constant resistance is placed in line with the grid and in parallel to an oscilloscope. With the use of oscilloscope, the current intensity can be calibrated in mA (milliamperes). The stimulus is typically applied over the paws of rodents. Rodents are kept in singly in plastic cages and electrical stimulus of accentuating intensities are

applied. The animals exhibits a immediate startling/flinching reaction. The reactions are recorded as variations in the displayed pulse. Difference in the threshold of pain before and after administration of drugs is measured. [60-62]

4.3.3.2.B Electrical stimulation of tail

Tail of mice is sensitive to any kind of stimulus. Electrical stimulation model has been first described by Burn et al. Variations in the duration or intensity of the electrical stimulus are used to observe the response. The effect of central analgesics and also the activity of peripheral analgesics at higher doses can be clearly demonstrated by this method. After keeping the mice in specialized cages, two alligator clips are positioned on the tail. At the proximal end, a positive electrode is kept on the tail. Waves with an voltage of 50 V and frequency of 1 shock per second are delivered from a constant voltage stimulator for duration of 2.5 milliseconds. Animal normally responds to the stimuli in 3 to 4 seconds. Following drug administration, the response time is observed and recorded every 15 minutes, till the reaction time is back to its baseline values. Ultrasonic stimulation may be used instead of electrical stimulus. The advantage of this method is that it is speedy, uncomplicated, and accurate. Repeated stimuli can be used without inducing tissue damage. [60-62]

4.3.3.2.C Tooth pulp stimulation test

This test was first used to assess central analgesic activity in rabbits. The tooth pulp of the rabbit is stimulated and are observed for characteristic responses like licking, biting, chewing and head flicking. The animals are anaesthetized with fentanyl or thipentone sodium administered intravenously. Using a dental drill, pulp chambers of the front two upper incisors are exposed. Electrical stimulus is applied over the pulp chambers using electrode that supply a current of 0.2 mA at a frequency of 50 Hz for one second duration. Characteristic reactions like licking, biting, chewing, head flicking follows which are regarded as pain threshold response. The intensity of threshold current, expressed in mV is used as an marker of the intensity and duration of analgesia. Those drugs which produce an increase of the threshold versus the initial control by a factor of 2 or more is considered to possess anti nociceptive effect. Dogs and cats can be used as an alternative to rabbits. [60-62]

4.3.3.2.D Monkey shock titration test

This test was introduced based on the studies showing correlation between analgesic activity of drugs in rodents and in humans. However, further experiments in higher animals like monkeys are imperative to confirm the mechanism of action and to ascertain the acceptable dose in humans. This model is not recommended for routine screening. [60-62]

After restraining the monkeys in chairs, electrodes are secured to the shaved portion of the tail. An electrical stimulus of 0-4 mA is applied through the electrodes with the help of a shocker. Now the monkeys are observed whether they are pressing a bar to interrupt the shock. This interruption of shock is considered as shock titration activity (reaction time). The shock titration activity (reaction time) is recorded using stopwatch before and after drug administration. Also the reaction time can be compared between drug treated groups and control groups. [60]

4.3.3.3 Models using chemical stimulus

Stimulation using chemicals is intensifying and lasts for long duration unlike electric stimulus. Moreover, once administered it is unavoidable. The recorded behavioral scores in animals were considered clinically relevant to the pain in humans. [60-62]

4.3.3.3.A Formalin test in rats

This test necessitate the administration of an irritant chemical like 5-10% formalin subcutaneously to rats. The formalin will induce a slow and long-lasting pain. The preferred site of injection is the dorsal potion of front paw. Following the administration of formalin the animals are secured in separate cages and observed for uncontrolled licking or biting the formalin injected paw. The reaction time is noted using a timer. All animals are evaluated immediately after the administration of chemical and at 30 mins and 60 mins. When a drug is said to have

analgesic activity when they protect the animals from advocating themselves to the painful reactions. Formalin-induced painful reactions can be evaluated using a 4-level scale : [60-62]

SCORES	BEHAVIOUR
0	normal posture
1	Injected paw on ground but not supporting the animal
2	Injected paw elevated
3	Animal licking or biting the injected paw.

4.3.3.3.B Writhing test

This test is also known by the name, "abdominal contortion test" or "stretching response test" because of the characteristic reflex stretching behavior observed in response to the administration of irritant chemicals into the peritoneal cavity. The chemicals commonly used to induce the painful stimulus are acetic acid (0.6%), phenylbenzoquinone (0.02%) and sodium chloride (4%). These noxious chemicals when administered, irritates the pain receptors in the peritoneum either directly or indirectly through inflammatory mediators like prostaglandins, leukotrienes and other cytokines. All animals in treatment group are

pretreated with the test drug while animals in the control group are given distilled water before administration of chemical stimulus. Then the animals are observed in separate cages for a period of 10 minutes and number of writhing responses are noted. Percentage of inhibition is calculated by the formula given below:

$$\frac{\text{Average writhes (Control)} - \text{Average writhes (Test)}}{\text{Average writhes (Control)}} \times 100$$

If the value of the calculated percent inhibition is less than 70 %, the drug at that particular dose is considered to have no significant analgesic activity. [60-62]

4.3.3.4 Models using mechanical stimulus

4.3.3.4.A Haffner's tail clip method

All centrally acting analgesics can be detected by this method. Rodents are generally used for this test. An artery clip is usually applied over the proximal part of the tail of the animal to induce an unpleasant mechanical stimulus. The animals react immediately by biting or nibbling the tail in proximity to the clip. Usually a cut-off period of 15secs is taken and all animals are observed till this time. The test and control group results are compared and determined whether any significance between the groups exists. Drug that are able to increase the reaction time show considerable analgesic activity. [60-62]

4.3.3.4.B Randall-selitto test

This test is a reliable method to evaluate analgesic activity of a test drug in rodents. Usually, a mechanical stimulus is applied on the hind paw of the animal using an analgesiometer. Using the analgesiometer accentuating pressure is applied over the plantar surface of the hind paw till a threshold level is reached. The threshold level is recognized by paw withdrawal reflex to complicated movements where the animals attempts to set free its trapped paw. The threshold for paw withdrawal reflex is evaluated in both control and test groups after administration of normal saline and test drug respectively. Both the threshold values are compared and analyzed to have statistical significance. Analgesic activity is exhibited by increase in the threshold paw withdrawal reflex reaction. [60-62]

4.4 Oxidative Stress

Oxidative stress is defined as the disparity between free radicals generation and body's capacity to eliminate them. The free radicals are also referred to as reactive oxygen species because they are considered to be generated during oxygen metabolism. These cause widespread manifestations at cellular as well as molecular levels due to oxidative damages to lipid membranes and DNA. Homeostatic mechanisms in our body play a major role in the detoxification of these reactive species and is not necessarily a hazard to the body. However, if these reactive species are produced at a rate exceeding its removal then the homeostatic mechanisms fails and the body succumbs to

oxidative stress. As a result, the excess free radicals can confront with the biological membranes, cellular components and macromolecules, thus promoting various diseased states. Oxygen derivatives (superoxide anion, hydroxyl radical and hydrogen peroxide) and reactive nitrogen species (nitric oxide and peroxynitrite) are the most important free radicals involved in various disease states like cardiovascular, autoimmune, diabetes mellitus, cancers, infectious and inflammatory conditions and nervous system disorders.[63]

Homeostatic mechanisms in our body has an array of antioxidant enzymes that boost up the removal of the free radicals that are normally generated. Some important antioxidant enzymes present in our body are glutathione peroxidase, superoxide dismutase and catalase. For the optimum activity of these enzymes they need some co-factors namely selenium, zinc, iron, copper and manganese.[64]

Antioxidants prevent free radical induced tissue damage by either preventing their formation, scavenging them, or by accelerating their degradation. They can be categorized into enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin), transition metal binding proteins (tranferrin, ferritin, lactoferrin) and chain breaking antioxidants. The chain breaking antioxidants are further classified into lipid phase (tocopherols, carotenoids, flavonoids, ubiquinol) and aqueous phase (ascorbate, urate, glutathione and other thiols). Glutathione peroxidase, catalase, and superoxide dismutase are considered to be the three primary

enzymes implicated in direct removal of reactive oxygen species like hydroxyl radical, superoxide radical and hydrogen peroxide. Glutathione reductase, glucose-6-phosphate dehydrogenase, and cytosolic glutathione S-tranferases are considered as secondary enzymes which maintains a constant supply of glutathione and NADPH to optimize the function of the primary enzymes. Plant-derived medicines are good sources of natural antioxidants like carotenoids, ascorbic acid, α- tocopherol, flavonoids, and phenolics.[65]

4.4.1 Role of oxidative stress in epilepsy

Epilepsy is a highly prevalent serious brain disorder, and oxidative stress is regarded as a possible mechanism involved in epileptogenesis. Experimental studies suggest that oxidative stress is a contributing factor to the onset and evolution of epilepsy. Brain is considered to be highly susceptible to oxidative damage due to high lipid content. Animal studies as seizure models have provided evidence of increased oxidative stress (increased lipid peroxidation and protein carbonylation) following seizures. The free radicals produced due to oxidative stress may induce seizure activity either by increasing the concentration of excitatory neurotransmitter glutamic acid or by a decrease in the brain cortex content of inhibitory neurotransmitter GABA.[66]

In the central nervous system, enzymatic and non enzymatic antioxidants play a significant role in preventing oxidative damage. Animal studies in which antioxidants were used in addition to anticonvulsants showed decreased oxidative stress and reduced frequency of seizures. The

most extensively studied endogenous antioxidant in animal models of epilepsy is melatonin. Melatonin is a pineal gland hormone and well-known free radical scavenger whose basal level decreases in the saliva of epileptic patients.[67]

Evidences from various preclinical and clinical studies suggest anti oxidant therapies have the potential for neuroprotection in epilepsy that targets oxidative stress.[66-67]

4.4.2 Role of oxidative stress in pain

The pathophysiology of pain has a linear association with oxidative stress. Many studies have proved the presence of increased reactive oxygen species in pain syndrome. Cordero et al. [68] proved the important role of oxidative stress in the pathogenesis of fibromylagia. In fibromylagia patients, they found a disparity in the levels of coenzyme Q (CoQ) between plasma and cells. Increased CoQ levels was observed in plasma in contrast to cells. Thus CoQ levels were very low in blood mononuclear cells (BMCs). Moreover, these BMCs were demonstrated to have high levels of free radicals. Thus, fibromylagia can be considered as an oxidative stress state. Further, they treated these patients with antioxidants like CoQ and tocopherol. They observed a significant reduction in the free radicals level in these patients.

Inamr et al.[69] done a study on 137 patients with acute and chronic inflammatory or non-inflammatory back pain. They found out that the

malondialdehyde (MDA) levels were significantly higher in patients when compared to controls. The increased MDA levels represent oxidative stress.

Evidences from various studies suggest that oxidative stress is implicated in the pathophysiology of pain and antioxidant therapy by decreasing the free radicals have significant nociceptive effect.

METHODOLOGY

5. Material and Methods

5.1. Study design

Experimental *in vivo* study in animals

5.2. Study setting and duration

The study was conducted over a time period of six months (December 2014 – June 2015) at the Research laboratory, Department of Pharmacology, Sree Mookambika Institute of Medical Sciences [SMIMS], Kulasekharam, Kanyakumari District, Tamilnadu.

5.3. Ethics committee approval

The study was approved by the Institutional Animal Ethics Committee with reference number SMIMS/IAEC/2014/C/02 [dated 29/09/2014].

5.4. Animals used

5.4.1. Species

Healthy adult Wistar albino rats of either sex, weighing between 150-250 g were selected for the study. A total of 72 animals were obtained from the central animal house, Sree Mookambika Institute of Medical Sciences [SMIMS], Kulasekharam.

5.4.2. Housing conditions

Adult Wistar albino rats of either sex were maintained in the central animal house of this institute as per the Committee for the

Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines. Male and female rats were housed in separate cages. Pregnancy was excluded among the female rats before selection for the study. All the animals which were handles with care to minimize stress and pain to them. The animals were given about one week for acclimatization to the new environment before the experiment was started. During this period they were provided with standard commercial rodent diet, water and the rooms were maintained at 27±2^{0}C , 70-80% humidity and 12 hour light/dark cycle. All experiments were conducted between 9:00 AM and 3:00 PM to minimize the variations in the data obtained.[70]

5.5. Drugs and chemicals used

A. Vehicle control – Sterile water

B. Test drug – Ethanolic extract of *Elettaria cardamomum* seeds. Doses used were 200 mg/Kg and 400 mg/Kg BW. The doses were selected based on the previous studies done with *Elettaria cardamomum* seeds.[6,11]

C. Standard drugs

- **For pain models -** Morphine (Morphine sulfate – 10 mg/mL; MORPHITROY 10; Troikaa Pharmaceuticals Ltd., Gujarat, India)

- **For MES model -** Pheytoin (Phenytoin sodium – 50 mg/mL; EPTOIN; Akums Drugs and Pharmaceuticals Ltd., Haridwar, India)
- **For PTZ model -** Sodium Valproate (Valproic acid – 100 mg/mL; ENCORATE; Unimed Technologies Ltd., Gujarat, India**)**

D. Drugs used to induce writhing [60-62]

- **0.6% Acetic acid –** Acetic acid (Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India) in volume 10 ml/Kg BW was used.

E. Drugs used to induce convulsions [60-62]

- **Pentylenetetrazole (PTZ) – (**Sigma Aldrich, USA)

F. Other drugs [60-62]

- **Lignocaine** (Lignocaine Hydrochloride injection; LOX 2%; VHB Medi Sciences Limited, Uttarakhand, India)

5.6. Equipments used

Electroconvulsiometer (MSK Private Ltd. Chennai, India), stop watch, beakers (100mL, 500mL), stirrers, spatula, china dish, conical flask, water bath, fine muslin filter cloth, Whatmann filter paper, autoclave, refrigerator, airtight sterile container, electronic weighing machine, syringes (1, 3, 5 mL), oral feeding needle.

5.7. Preparation of seed extract

5.7.1. Identification and authentication of *Elettaria cardamomum* seeds

Elettaria cardamomum seeds were procured from the commercial local market in Kulasekharam, Tamil Nadu. The *Elettaria cardamomum* seeds were authenticated by Bioscientist and head, School of Biosciences, Mar Athanasios College for Advanced studies (MACFAST), Tiruvalla, Kerala. The authenticated plant seeds were then kept as a specimen in the Museum, Department of Pharmacology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari District, Tamil Nadu.

5.7.2. Preparation of dry seed powder

The collected *Elettaria cardamomum* seeds were washed with running water and dried. The dried seeds were grounded using an electric mixture to obtain a coarse powder.

5.7.3. Solvent used

Ethanol (99.9%, Hong Yang Chemical Corp., China)

5.7.4. Preparation of ethanolic extract of *Elettaria cardamomum* seeds

The ethanolic extract of Elettaria cardamomum seeds was prepared by cold percolation method. Dried powdered seeds were weighed using electronic weighing balance. 100 g of the dried seed powder was taken and it was soaked in 500 mL of ethanol solution in a

conical flask. The solution with its contents were stirred well and kept for 7 days with intermittent shaking. On the 8th day the contents were filtered using Whatman's filter paper and excess solvent was allowed to undergo evaporation without applying heat. The contents which remained in the filter paper were transferred into a conical flask and mixed with 99.9% ethanol solution and was used for the preparation of second batch of the extract using the same procedure as mentioned above. A semi solid dark brown extract was obtained after evaporation. The two batches of the extracts were mixed together and the whole extract was weighed. The obtained extract was heat sterilized in n autoclave at 121^0C for thirty minutes. The extract was transferred to an airtight sterile container and was refrigerated for further use in the study. Required concentrations of the extract were freshly prepared just before the experiment by weighing appropriate amount of the stored extract and diluting it with sterile distilled water.[71]

5.7.4.1. Yield

For 100 g of dry seed powder, 30.5 g of the condensed extract was obtained. (Yield = 30.5%)

5.8. Study procedure

The experiments were carried out as per the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on

Animals (CPCSEA). An acclimatization period of one week was given before starting the study. All the animals were handled with utmost care to minimize the stress and pain. The animals used for the experiments were fasted overnight with free access only to water. They were properly grouped and marked to avoid mixing of groups.

5.8.1. Grouping of animals

The animals used for the study were divided into twelve groups [Group I to Group IV; Group A to Group D; Group 1 to Group 4], each consisting of six animals. A total of 72 animals were used for this study. Of which, 48 animals were used for evaluating anticonvulsant activity and 48 animals were used to evaluate analgesic activity. Same animals [Groups 1 to 4] were used to evaluate analgesic activity sequentially using two models (Tail warm water immersion test followed by acetic-acid induced writhing test) to reduce the number of animals used in the study.

Table 1. Grouping of animals for evaluating anticonvulsant activity[55]

Electrically induced convulsions (With current 150 mA, at frequency 60 Hz, for duration 0.2 seconds)	
Group I	Sterile water equivalent volume orally [Vehicle control]
Group II	Phenytoin 25 mg/Kg BW intraperitoneally [Positive control]
Group III	Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)
Group IV	Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

Pentylenetetrazole (70 mg/Kg BW, s.c) induced convulsions	
Group A	Sterile water equivalent volume orally [Vehicle control]
Group B	Sodium valproate 400 mg/Kg BW intraperitoneally [Positive control]
Group C	Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)
Group D	Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

n=6 in each group

BW - Body weight; s.c - subcutaneous; mA - Milliamperes; Hz - Hertz

Table 2 : Grouping of animals for evaluating analgesic activity [60]

Analgesic Activity	
Group 1	Sterile water equivalent volume orally [Vehicle control for both tail immersion and writhing tests]
Group 2	Morphine 5 mg/Kg BW intraperitoneally [Positive control for tail immersion and writhing tests]
Group 3	Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)
Group 4	Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

n=6 in each group; BW – Body Weight

5.8.2. Evaluation of anticonvulsant activity

5.8.2.1. MES model

The grouping of animals is shown in Table 1. The test drug (Ethanolic seed extract of *Elettaria cardamomum* – 200 mg/Kg BW and 400 mg/Kg BW) was given orally and standard drug (phenytoin–25mg/Kg) was injected intraperitoneally (i.p). 30 minutes after i.p injection and 60 minutes after oral administration, corneal electrodes were used to induce seizures. Before placing the

electrodes, the eyes of the rats were moistened and anesthetized with lignocaine (2% solution). The electroconvulsiometer was set to deliver a stimulus of 150 mA intensity with a frequency of 60 Hz for duration of 0.2 seconds. Tonic hind limb extension (present/absent) and seizure activity scoring were observed for each animal.[54-56]

5.8.2.2. PTZ model

The grouping of animals is shown in Table 1. Each animal was weighed individualy. The test drugs (Ethanolic extract of *Elettaria cardamomum*– 200 mg/Kg and 400 mg/Kg) were given orally and standard drug (Sodium valproate–400 mg/Kg) was injected intraperitoneally (i.p). 30 minutes after i.p injection and 60 minutes after oral administration, pentylenetetrazole was given in a dose of 70 mg/Kg subcutaneously. Each animal was then placed separately and observed for one hour. Seizures and tonic convulsions were recorded. For each animal, time for onset of seizure, total number of seizures in one hour and duration of each seizure were noted. The total duration of seizure was obtained by adding up the duration of each seizure for an animal. The severity of seizures was also scored for each animal. [54-56]

5.8.3. Evaluation of analgesic activity

A total of four groups each consisting of six animals were used in the evaluation of analgesic activity. Each animal from groups 1 to group 4 was individually tested in a sequential manner using two analgesic

models. Thermal stimuli induced pain model - Tail warm water immersion test, was done first to evaluate the central analgesic activity. The same animals were then subsequently the next day, subjected to chemical stimuli induced pain model - writhing test, for testing peripheral analgesic activity.[60-62]

5.8.3.1. Tail Warm Water Immersion test

Procedure: The rats were placed into separate cages with their tail hanging out freely. The distal 5cm part of the tail was marked. The test drug ethanolic extract of *Elettaria cardamomum* seeds (in 200, 400 mg/kg BW orally) and standard drug Morphine (5mg/kg BW intraperitoneally) were administered to the corresponding groups as shown in Table 2. Thirty minutes after intraperitoneal administration and sixty minutes after oral administration of the above drugs, the distal part of the tail was immersed in a beaker filled with warm water (Temp=55^{o}C ; max upto 15 secs)

Response: A tail withdrawal reflex was observed within a few seconds.

Evaluation: The exact latency to react (time between stimulation onset and response) in seconds, was observed using stop watch and noted. [60-62]

5.8.3.2. Writhing test

Procedure: Standard (Morphine - 5 mg/Kg BW; intraperitoneally and test drugs (ethanolic extract of *Elettaria cardamomum* – 200

mg/Kg BW and 400 mg/Kg BW) were administered orally to animals of the corresponding groups as shown in Table 2. Thirty minutes after intraperitoneal drug administration and sixty minutes after oral drug administration, writhing was induced with 0.6% acetic acid (10 mL/Kg) which was injected intraperitoneally. Each rat was individually observed for the writhing response.

Response: Writhing response was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Evaluation: The time for onset of writhes and the number of writhes in each animal was observed for a period of 30 minutes. The percentage of inhibition was also calculated. [60-62]

5.8.4. Parameters

A. ANTICONVULSANT ACTIVITY:

i. Maximal electroshock seizure (MES) model [54-56]

- Presence or Absence of Tonic Hind Limb Extension
  - Tonic Hind Limb Extension is a position when tail and both hindlimbs are parallel to each other during an episode of generalized tonic clonic seizure.
- Seizure activity scoring (Score 0-4) as shown below:
  - 0 – No seizure
  - 1 – Forelimb extension without hindlimb extension
  - 2 – Complete forelimb extension and partial hindlimb extension

- 3 – Complete tonic hindlimb extension
- 4 – Postictal depression

♦ Percentage protection (%) calculated as follows

$$\frac{\text{Number of animals with THLE absent}}{\text{Total number of animals}} \times 100$$

ii. Pentylenetetrazole (PTZ) model [54-56]

♦ Onset of seizure activity (in seconds)

♦ Total duration of seizure (in seconds)

♦ Number of seizures in one hour

♦ Severity of seizures[53] (Score 0-5) as shown below

- 0.5 – Atypical behavioral changes (intense grooming, sniffing, moving arrests)
- 1 – Isolated myoclonic jerks, ear and facial twitching
- 2 – Atypical minimal seizures, convulsive wave through the body
- 3 – Fully developed minimal seizures, clonus of the head muscles and fore limbs, righting reflex present
- 4 – Major seizures (generalized without tonic phase)
- 5 – Generalized tonic-clonic seizures beginning with running

B. <u>ANALGESIC ACTIVITY:</u>

i. Tail warm water immersion test [60-62]

- Reaction time in seconds (time between onset of stimulus and response)

ii. Writhing test [60-62]

- Number of writhes in 30 minutes
- Percent inhibition was calculated by the formula given below

$$\frac{\text{Average writhes (Control)} - \text{Average writhes (Test)}}{\text{Average writhes (Control)}} \times 100$$

5.9. Statistical analysis

- The data were entered into the Microsoft Office Excel 2007 for Windows 8.
- Data analysis was done using Statistical Package for Social Sciences (SPSS 16.0) version, 64 bit for Windows.
- One-way ANOVA [Parametric test] followed by Dunnet's post hoc test was used for data with normal (Gaussian) distribution to find out the statistical significance between the study groups
- $p < 0.05$ was considered as statistically significant
- The results in bar diagrams are presented as Mean± SD [For all other parameters]

RESULTS

6. Results:

6.1. Animals used in study:

Seventy two (72) Wistar strains of Albino rats of either sex were used in the study. All the animals were divided into twelve (12) groups of six (6) animals in each group. The grouping of animals has been shown in Table no: 1 and 2.

6.2. Assessment of mean scores of seizures in MES model: (Figure 1)

Group-II, III and IV showed significant ($p<0.05$) decrease in mean scores of seizures compared with Group-I. Group-II also showed significant ($p<0.05$) reduction in scores of seizures compared to Group-III but not with group-IV. Low dose of plant extract showed lesser effect compared to high dose it was statistically significant ($p<0.05$).

6.7. Assessment of the percentage of protection of Tonic Hind Limb Extension [THLE] in MES model: (Figure 2)

Rats given standard and two graded doses of test drug showed 100 % protection in MES induced THLE. But in the control group all the rats were showed THLE.

6.8. Assessment of onset of seizures in pentylenetetrazole model: (Figure 3)

Significant ($p<0.05$) increase in onset of seizure time was observed in rats pretreated with standard and high dose of test drug compared to control group. No significant ($p>0.05$) difference was observed compared group-B with Group-D. Low dose of plant extract treated rats showed less effect compared to standard and high dose test drug, it was statistically significant ($p<0.05$).

6.9. Assessment of duration of seizures in pentylenetetrazole model: (Figure 4)

Pretreatment with standard and graded doses of test drug showed significant decline in mean duration of seizure time compared to group-A. Maximum reduction in duration of seizures was observed in Group-B compared to Group-C and D, it was statistically significant ($p<0.05$).

Rats pretreated with high dose of plant extract showed better efficacy compared to low dose. The difference between two groups was statistically significant ($p<0.05$).

6.10. Assessment of number of seizures [in 1 hour] in pentylenetetrazole model: (Figure 5)

Standard and test drugs given rats showed less number of seizures compared to control group ($p<0.05$). Maximum protection was observed with standard and high dose of test drug given rats compared to low dose test drug given rats.

No statistical significant difference was found between Group-B when compared to Group-D [$P>0.05$].

6.11. Assessment of scores of seizures in pentylenetetrazole model: (Figure 6)

Prior administration of standard and high dose test drug groups showed significant ($p<0.05$) reduction in the mean scores of seizures compared to control group. High dose test drug showed similar results that of the standard group ($p>0.05$). Low dose of plant extract given rats showed less protection compared to high dose.

6.2. Assessment of reaction time in tail warm water immersion test for evaluation analgesic activity: (Figure 7)

Maximum analgesic effect was observed in rats pretreated with morphine and high dose of test drug compared to control group, it was statistically significant ($p<0.05$). The increase in reaction time in Group 4 was comparable to Group 2, as no statistically significant difference was found between these two groups [$p>0.05$]. Significant ($p<0.05$) difference was observed between low and high dose of test drug in increasing the reaction time.

6.4. Assessment of total number of writhes [in 30 minutes] in 0.6% acetic acid induced model for evaluation of analgesic activity: (Figure 8)

Acetic acid induced writhes were significantly ($p<0.05$) reduced in group-2, 3 and 4 compared to group-I. Maximum protection was observed in group-2 and 4 compared to group-3, it was statically significant ($p<0.05$). Similar results were observed in rats treated with standard and high dose test drug and it was not statically significant ($p>0.05$). Less protective effect was observed in low dose test drug given rats compared to high dose.

6.5. Assessment of percent inhibition of total number of writhes [in 30 minutes] in 0.6% acetic acid induced model for evaluation of peripheral analgesic activity: (Figure 9)

The percent inhibition of total number of writhes [in 30 minutes] in different groups includes Group 1 [0%], Group 2 [97.81%], Group 3 [72.71%] and Group 4 [90.28 %].

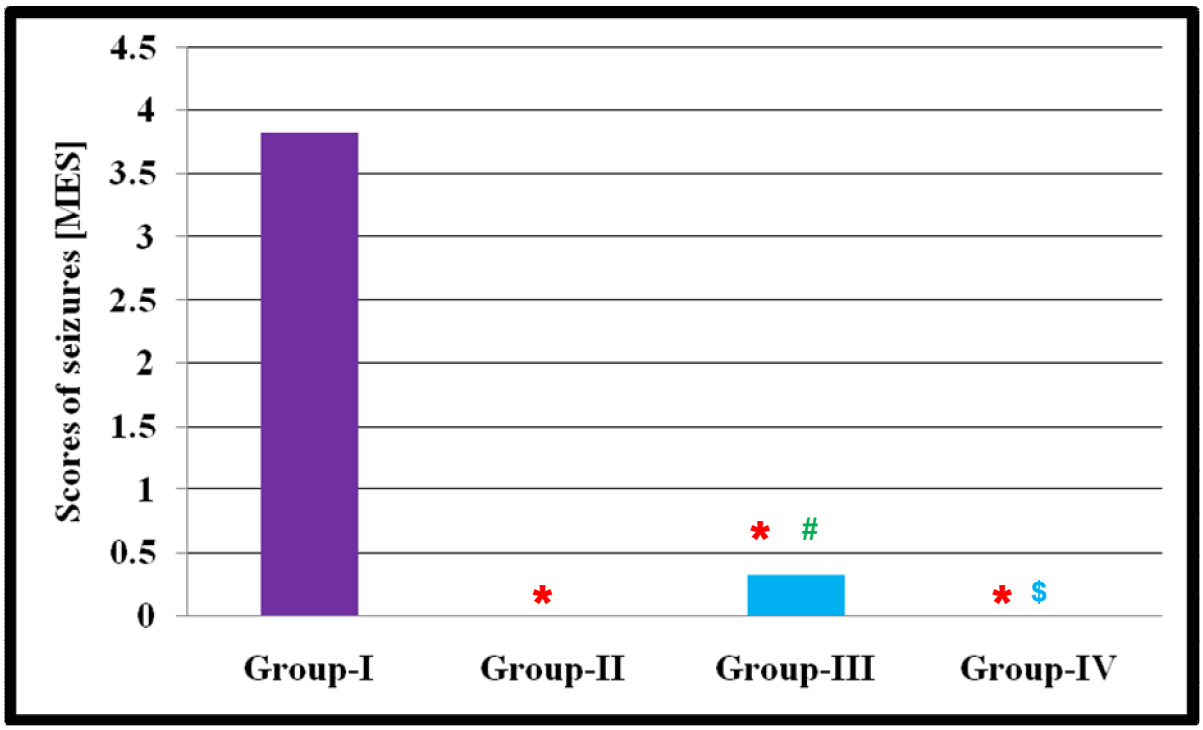

Figure 1. Bar diagram showing the scores of seizures in Maximal Electroshock Seizure test in Wistar Albino rats

Data are represented as **Median± SD**

n = 6 in each group

*p < 0.05 when compared to group I

#p < 0.05 when compared to group II

$p < 0.05 when compared to group III

Group I: Sterile water equivalent volume orally [Vehicle control]

Group II: Phenytoin 25 mg/kg body weight intraperitoneally [Positive control]

Group III: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg body weight orally)

Group IV: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg body weight orally)

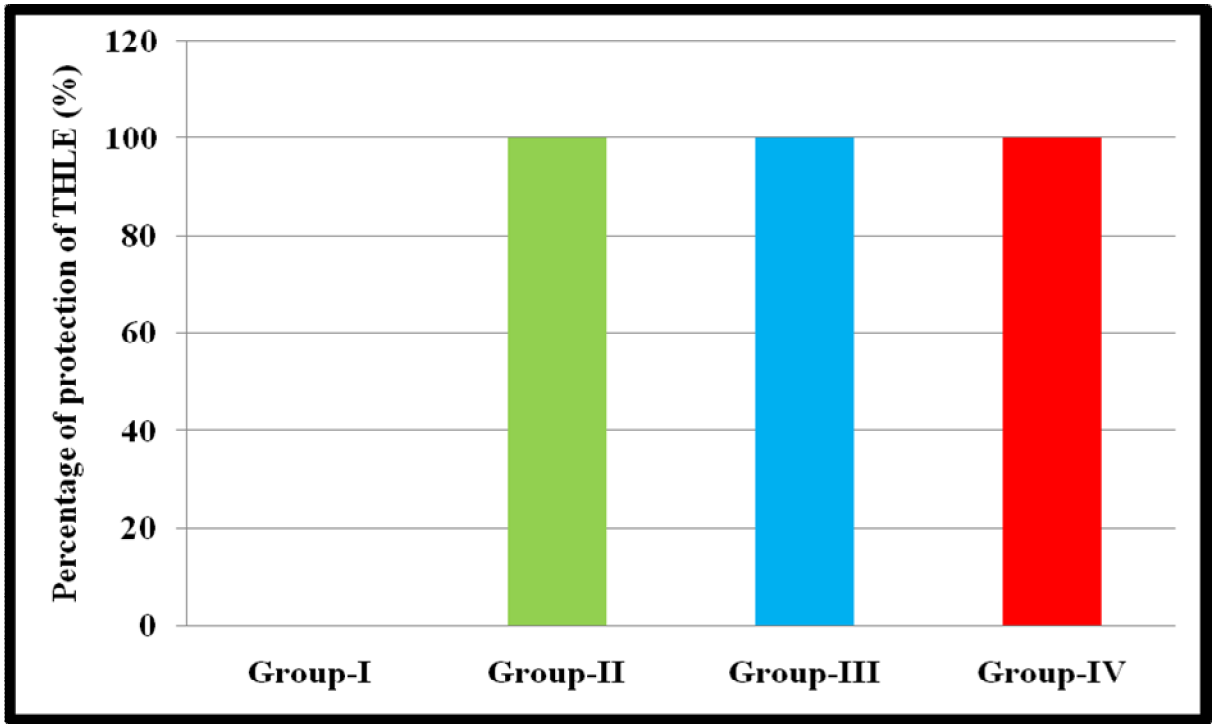

Figure 2. Bar diagram showing the percentage of protection of Tonic Hind Limb Extension [THLE] in Maximal Electroshock Seizure test in Wistar Albino Rats.

Data are represented as **Percentage [%]**

n = 6 in each group

Percentage protection was calculated by $\frac{\text{Number of animals with THLE absent}}{\text{Total number of animals}} \times 100$

Group I: Sterile water equivalent volume orally [Vehicle control]

Group II: Phenytoin 25 mg/kg body weight intraperitoneally [Positive control]

Group III: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg body weight orally)

Group IV: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg body weight orally)

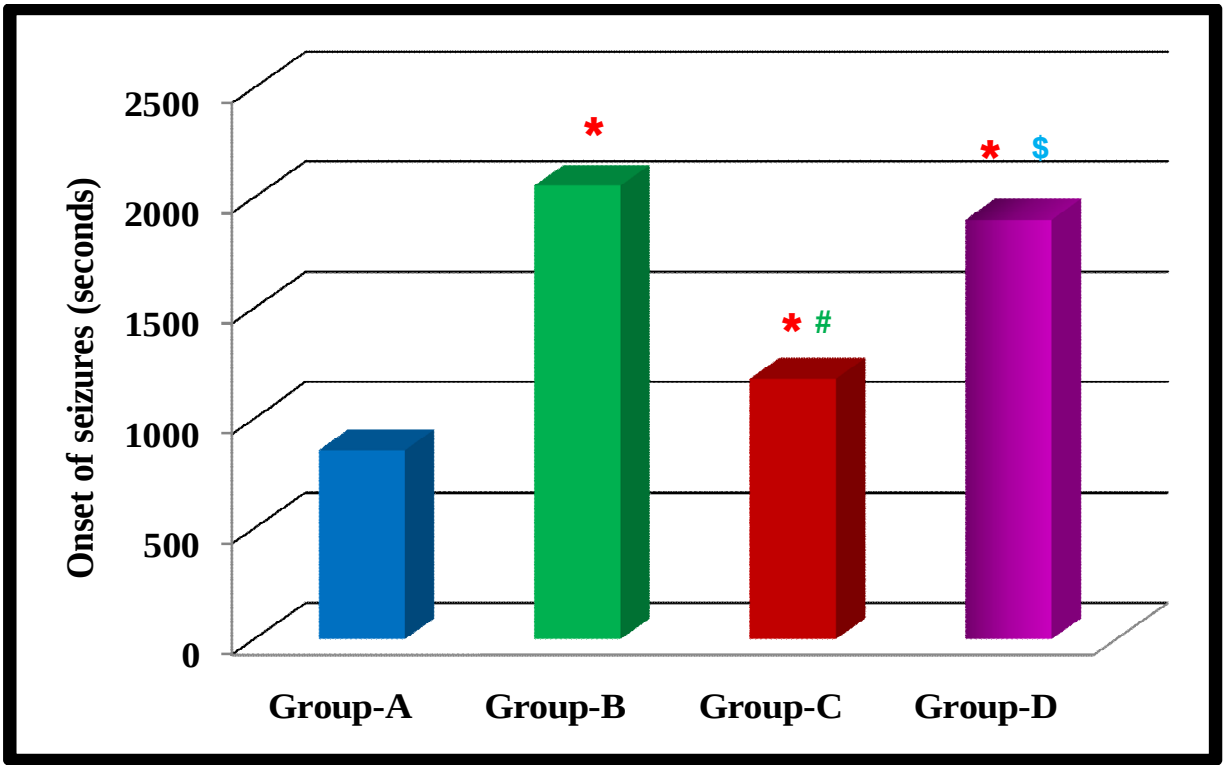

Figure 3. Bar diagram showing the onset of seizures (seconds) using Pentylenetetrazole(PTZ) test in Wistar Albino Rats.

Data are represented as **Mean ± SD**

n = 6 in each group

*p < 0.05 when compared to group A

#p < 0.05 when compared to group B

$p < 0.05 when compared to group C

Group A: Sterile water equivalent volume orally [Vehicle control]

Group B: Sodium valproate 400 mg/kg body weight intraperitoneally [Positive control]

Group C: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)

Group D: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

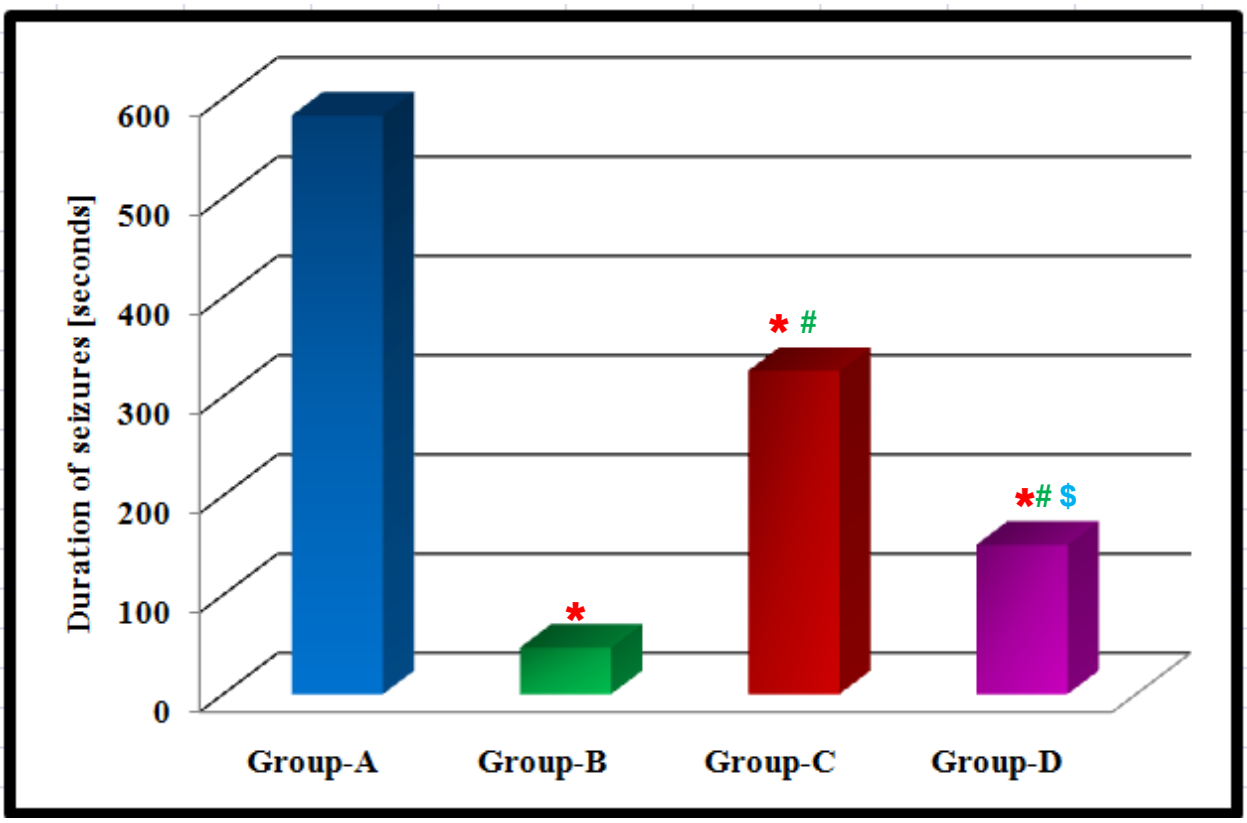

Figure 4. Bar diagram showing the duration of seizures (seconds) using Pentylenetetrazole(PTZ) test in Wistar Albino Rats.

Data are represented as **Mean ± SD**

n = 6 in each group

*p < 0.05 when compared to group A

#p < 0.05 when compared to group B

$p < 0.05 when compared to group C

Group A: Sterile water equivalent volume orally [Vehicle control]

Group B: Sodium valproate 400 mg/kg body weight intraperitoneally [Positive control]

Group C: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)

Group D: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

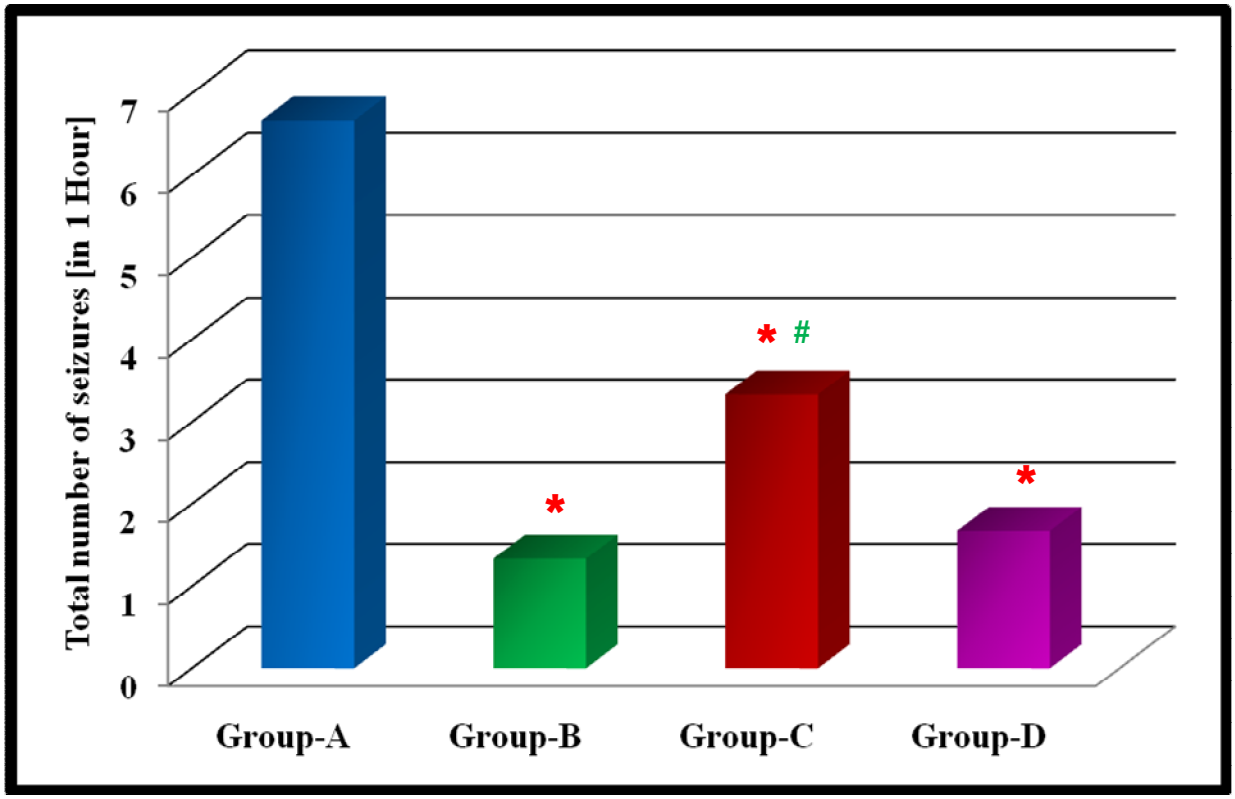

Figure 5. Bar diagram showing the total number of seizures (in 1 hour) using Pentylenetetrazole(PTZ) test in Wistar Albino Rats.

Data are represented as **Mean ± SD**

n = 6 in each group

*****p < 0.05 when compared to group A

#p < 0.05 when compared to group B

Group A: Sterile water equivalent volume orally [Vehicle control]

Group B: Sodium valproate 400 mg/kg body weight intraperitoneally [Positive control]

Group C: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)

Group D: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

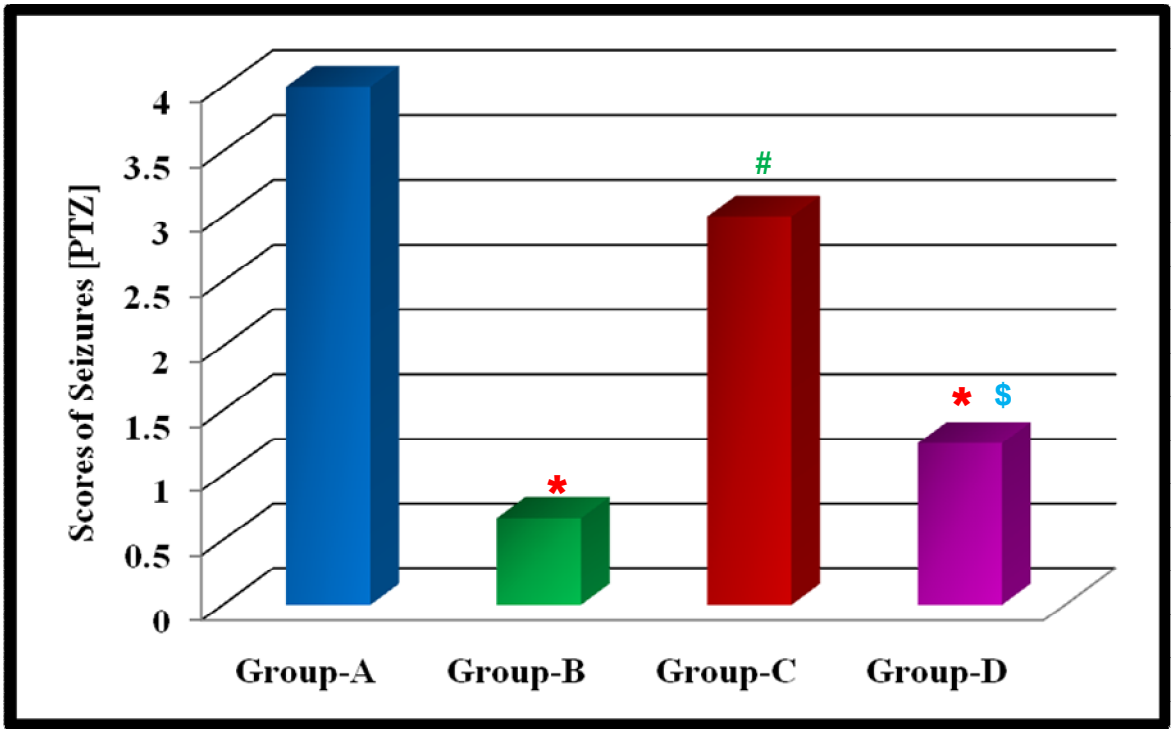

Figure 6. Bar diagram showing the scores of seizures using Pentylenetetrazole(PTZ) test in Wistar Albino Rats.

Data are represented as **Mean ± SD**

n = 6 in each group

***p** < 0.05 when compared to group A

$^{\#}$p < 0.05 when compared to group B

$^{\$}$p < 0.05 when compared to group C

Group A: Sterile water equivalent volume orally [Vehicle control]

Group B: Sodium valproate 400 mg/kg body weight intraperitoneally [Positive control]

Group C: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)

Group D: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

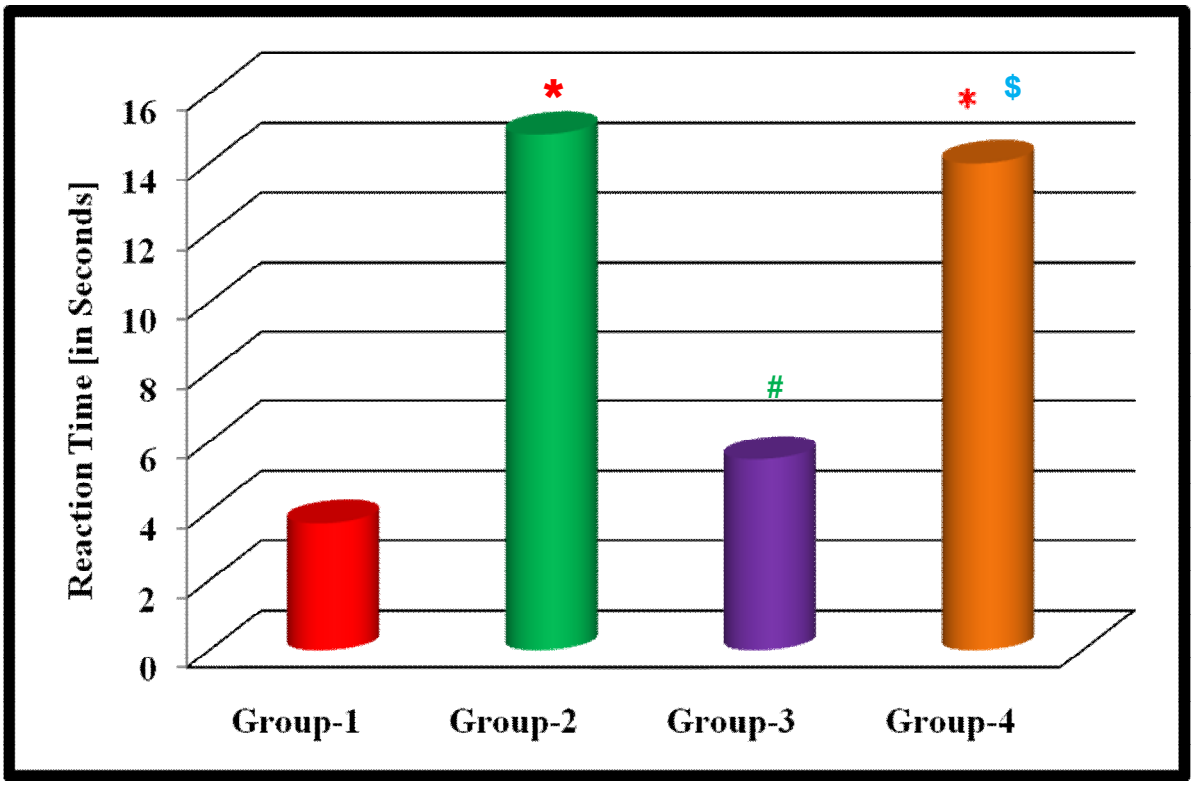

Figure 7. Bar diagram showing the reaction time [seconds] in tail warm water immersion test in Wistar Albino rats

Data are represented as **Mean ± SD**
n = 6 in each group
***p** < 0.05 when compared to group 1
[#]p < 0.05 when compared to group 2
[$]p < 0.05 when compared to group 3

Group 1: Sterile water equivalent volume orally [Vehicle control]

Group 2: Phenytoin 25 mg/kg body weight intraperitoneally [Positive control]

Group 3: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg BW orally)

Group 4: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg BW orally)

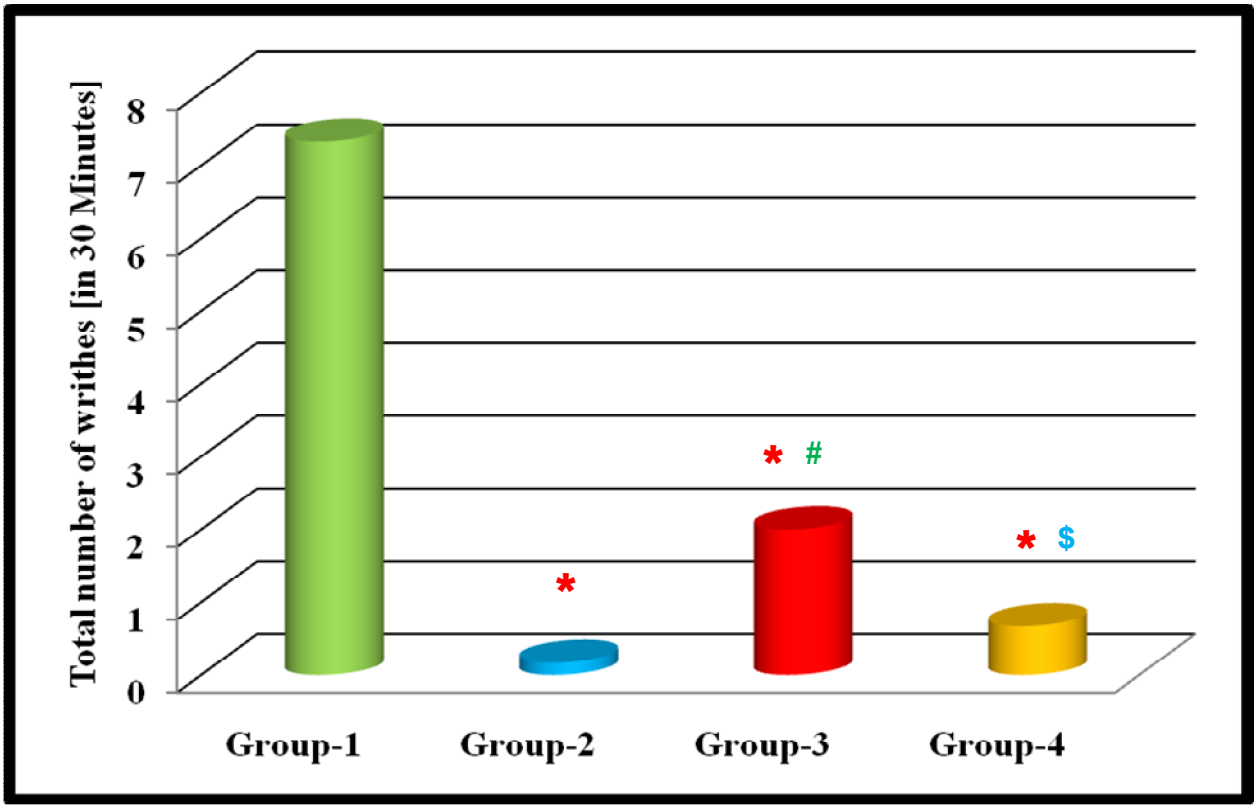

Figure 8. Bar diagram showing the total number of writhes in 30 minutes in 0.6% acetic acid induced model of analgesic activity among different groups in Wistar Albino rats

Data are represented as **Mean ± SD**

n = 6 in each group

*p < 0.01 when compared to group 1

#p < 0.01 when compared to group 2

$p < 0.01 when compared to group 3

Group 1: Sterile water equivalent volume orally [Vehicle control]

Group 2: Phenytoin 25 mg/kg body weight intraperitoneally [Positive control]

Group 3: Ethanolic extract of *Elettaria cardamomum* seeds (200 mg/Kg body weight orally)

Group 4: Ethanolic extract of *Elettaria cardamomum* seeds (400 mg/Kg body weight orally)

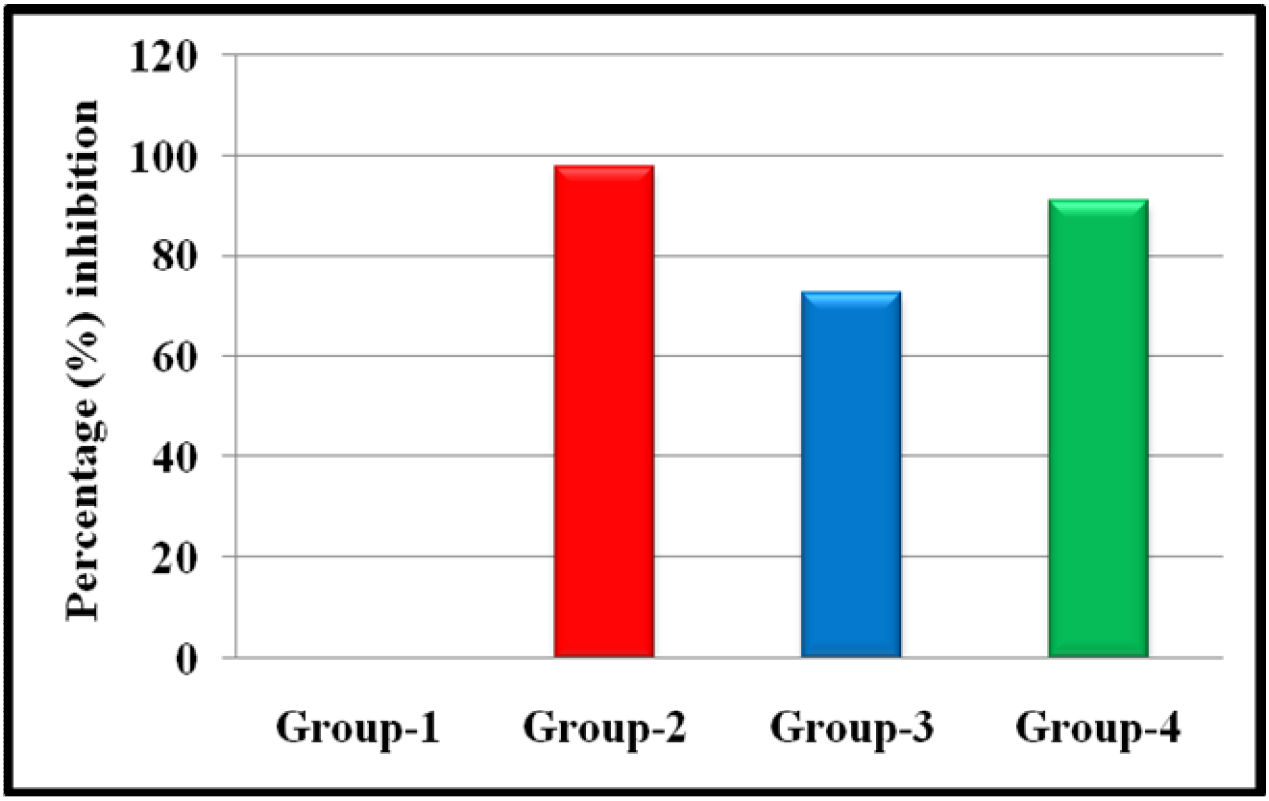

Figure 9. Bar diagram showing the percent inhibition of total number of writhes in 30 minutes in 0.6% acetic acid induced model of analgesic activity among different groups in Wistar Albino rats

n = 6 in each group

% Inhibition was calculated by $\frac{\text{Average writhes (Control)} - \text{Average writhes (Test)}}{\text{Average writhes (Control)}} \times 100$

Group 1: Sterile water equivalent volume orally [Vehicle control]

Group 2: Phenytoin 25 mg/kg body weight intraperitoneally [Positive control]

Group 3: Ethanolic extract of Elettaria cardamomum seeds (200 mg/Kg BW orally)

Group 4: Ethanolic extract of Elettaria cardamomum seeds (400 mg/Kg BW orally)

DISCUSSION

7. Discussion

The current study revealed that Ethanolic extract of *Elettaria cardamomum* seeds possessed anticonvulsant activity in MES and PTZ models in Wistar strains of albino rats. Tail warm water immersion test and acetic-acid induced writhing test were used to evaluate analgesic activity. There are several models used for screening anticonvulsant activity in animals. MES and PTZ models are the most accepted because convulsions produced represent generalized tonic-clonic and absence seizures in humans respectively.[54]

This study was done as per CPCSEA guidelines. All precautions to minimize bias due to gender or diurnal variations were taken. Male and female rats were equally distributed among the groups to minimize gender variations on the results. Pregnancy was excluded in female rats before inclusion into the study. All experiments were conducted during the same hours on all days to minimize diurnal variations. A total of 72 Wistar strains of albino rats were used to evaluate anticonvulsant and analgesic property of Ethanolic extract of *Elettaria cardamomum* seeds. The extract at a dose of 400 mg/Kg BW showed similar protective effects against convulsions and pain to the standard drugs used in the experiment.[70]

In MES model, both high and low doses of cardamom seed extract showed 100% protection against tonic hind limb extension (THLE). Moreover, significant reduction in seizure scores were observed with both

doses. In PTZ model, plant extract at both doses showed significant delay in onset of seizures and decreased the duration as well as total number of seizures. High dose of plant extract showed more efficacy than low dose. These results were similar to the study done by Gohain et al.[72] on ethanolic extract of seeds of *Benincasa hispida* Linn. in albino rats showing anticonvulsant activity with both MES and PTZ at 200 and 400 mg/kg. Achilya et al.[45] observed that pretreatment with polyherbal formulation containing *Elettaria cardamomum* seed powder at an oral dose of 300 mg/kg prevented MES and PTZ induced convulsions in mice. The similar protective effects were observed in the present study using 200 and 400 mg/kg of cardamom seed extract.

Analgesic activity was evaluated using central as well as peripheral models. Tail warm water immersion test and writhing test is considered as central and peripheral analgesic models respectively. In tail warm water immersion test, high dose of plant extract showed significant latency in reaction time which was comparable to standard group. In acetic acid-induced writhing test, the total number of writhes were decreased with both graded doses of the seed extract. The study results were consistent with another study done by Kumar et al.[16] Similar protective effects were observed with cardamom seed oil using p-benzoquinone induced writhing response in mice.

Central and peripheral oxidative stress is an important factor implicated in the pathogenesis of seizures and pain respectively. Antioxidant

potential of *Elettaria cardamomum* seeds has been proven by several studies. Phytochemical analysis with various extracts of cardamom revealed the presence of phenols. The phenolic compounds like flavonoids and tannins are important antioxidants derived from plants. Shetty et al.[28] in his study has confirmed the presence of phenols in the Ethanolic extract of cardamom seeds. Ashadevi et al.[18] showed remarkable anion scavenging activity of methanolic extract of cardamom seeds due to the presence of high concentration of phenolics. As phenols are effective hydrogen donors, they act as free radical scavengers and reduce the oxidative stress. In the present study with Ethanolic extract of cardamom seeds, the anticonvulsant and analgesic activity may be due to the presence of these phenolic compounds. Prabhu et al.[73] evaluated the analgesic activity of Ethanolic extract of leaves of *Tridax procumbens* (dose -200 mg/kg and 400 mg/kg) against acetic-acid writhing model in rats and proposed that the analgesic activity of the test drug may be attributed to the inhibition of cycloxygenase and/or lipoxygenase in the periphery, thereby decreasing synthesis of prostaglandins and leukotrienes. Similar mechanism of action can also be suggested for Ethanolic extract of *Elettaria cardamomum* seeds.

The study had the following limitations. The chemical constituents of the ethanolic extract of *Elettaria cardamomum* seeds were not isolated and tested individually for its efficacy. MES and PTZ models are acute seizure models.[55] Since epilepsy is a chronic condition requiring daily drug administration. The reduction of oxidative stress was proposed to be the

cause of anticonvulsant and analgesic activity but the oxidative stress markers in blood were not estimated.

Further studies are required after isolating pure chemical constituents responsible for the anticonvulsant and analgesic activity of the Ethanolic extract of cardamom seeds. Future screening tests are required with higher animals.

CONCLUSION

8. Conclusion

Ethanolic extract of *Elettaria cardamomum* seeds possess significant anticonvulsant activity in Wistar albino rats at the dose of 400 mg/Kg BW.

Significant analgesic activity was observed in Wistar albino rats ethanolic extract of *Elettaria cardamomum* seeds at the dose of 400 mg/Kg BW.

SUMMARY

9. Summary

Herbal drugs are being researched and explored for wide therapeutic potentials. These phytomedicines have an outstanding place in modern therapeutics.[1] The hunt for newer anticonvulsants and analgesics continue due to the inherent constraints with the available drugs. *Elettaria cardamomum* commonly called cardamom is seen exuberantly in the Western Ghats forests of India. Traditional systems of medicine practiced in India use the seeds of this plant for various ailments including epilepsy and pain. The cardamom seeds are proved to have different medicinal properties that might contribute to its efficacy in the management of epilepsy and pain. The antioxidant property of the cardamom seeds needs special mention as recent literature suggests a linear relationship between the pathogenesis of epilepsy and pain with excessive free radicals. This study was aimed to evaluate the anticonvulsant and analgesic activity of ethanolic extract of *Elettaria cardamomum* seeds in Wistar Albino rats.

Seventy two (72) Wistar albino rats of either sex weighing around 150-250 g were selected for this study. The ethanolic extract of *Elettaria cardamomum* seeds were prepared in two doses - 200mg/kg BW and 400mg/kg BW and administered to the animals in the test groups. The anticonvulsant property was evaluated using two models - MES and PTZ which is an electrically and chemically induced seizure models respectively. In MES test, both the doses showed 100% protection of tonic hind limb and high dose exhibited a reduction of the seizure scores similar to the standard

drug used. In PTZ test, high dose of plant extract was demonstrated to have prolonged onset, decreased duration and total number of seizures almost identical to the standard drug administered. Significant anticonvulsant activity was shown by Ethanolic extract of *Elettaria cardamomum* seeds at a dose of 400mg/kg BW in Wistar albino rats.

In tail warm water immersion test, morphine was used as the standard drug to compare the analgesic property of plant extract. The prolongation of the reaction time in animals pretreated with high dose of plant extract was identical to those treated with morphine. This test demonstrated the central analgesic property of Ethanolic extract of *Elettaria cardamomum* seeds in Wistar albino rats. In acetic acid induced writhing test, rats pretreated with high dose of plant extract showed similar efficacy in reducing the number of writhes compared to the standard drug. This test confirmed the peripheral analgesic property of Ethanolic extract of *Elettaria cardamomum* seeds in Wistar albino rats.

Phenolic compounds have been isolated from various extracts of cardamom seeds. These compounds are known free radical scavengers and reduce oxidative stress. Since oxidative stress is implicated in the pathogenesis of epilepsy and pain, the phenolics present in the Ethanolic extract of cardamom seeds might be responsible for anticonvulsant and analgesic property. Recently, phenolics have been found to modulate $GABA_A$-Cl channels which might also contribute for its anticonvulsant potential.

This study concluded with the findings that Ethanolic extract of *Elettaria cardamomum* seeds have significant anticonvulsant and analgesic activity in Wistar albino rats. Further studies in higher animals using pure chemical constituents isolated from the extract is necessary to support the evidence.

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ANNEXURES

Annexure 1 : Institutional Animal Ethics Committee Certificate

Sree Mookambika Institute of Medical Sciences
Kulasekharam (K.K District, TN) 629161
Phone No: 04651-280866, Fax No. 04651-280740

Institutional Animal Ethics Committee
Registered under CPCSEA with Reg No. 1144/ac/07/CPCSEA

Ref. No. SMIMS/IAEC/2014/C/02 Date: 29th September 2014

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IAEC/2014/C/02,** entitled "Evaluation of Anticonvulsant and Analgesic Properties of Ethanolic Extract of *Elettaria cardamomum* Seeds in Wistar Albino Rats" submitted by Dr. Arjun G Nair, Postgraduate of Department of Pharmacology, SMIMS has been approved by the Institutional Animal Ethics Committee at its meeting held on 29th of September 2014.

[This Institutional Animal Ethics Committee is organized and operates according to the requirements of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Guidelines, Ministry of Environment and Forests, Government of India.]

IAEC Date 29/09/14

Dr. Vijay Pal Bhalla
Member Secretary
Institutional Animal Ethics Committee
SMIMS, Kulasekharam [K.K District]

Dr. J. C Stephenson
Member and CPCSEA Main Nominee
Institutional Animal Ethics Committee
SMIMS, Kulasekharam [K.K District]

Annexure 2: *Elettaria cardamomum* plant

Annexure 3: Authentication of *Elettaria cardamomum* seeds

Certificate of Botanical Authenticity

Certified that the plant specimen has been identified as ***Elettaria cardamomum* (L.) Maton** belonging to the family ***Zingiberaceae***

Name of the plant	**:**	***Elettaria cardamomum* (L.) Maton**
Part used	**:**	**Seed**
Family	**:**	***Zingiberaceae***
Loclocality	**:**	**Trivandrum, Kerala**
Collected by	**:**	**Dr. Arjun.G.Nair**

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Biju Dharmapalan

HEAD OF DEPARTMENT
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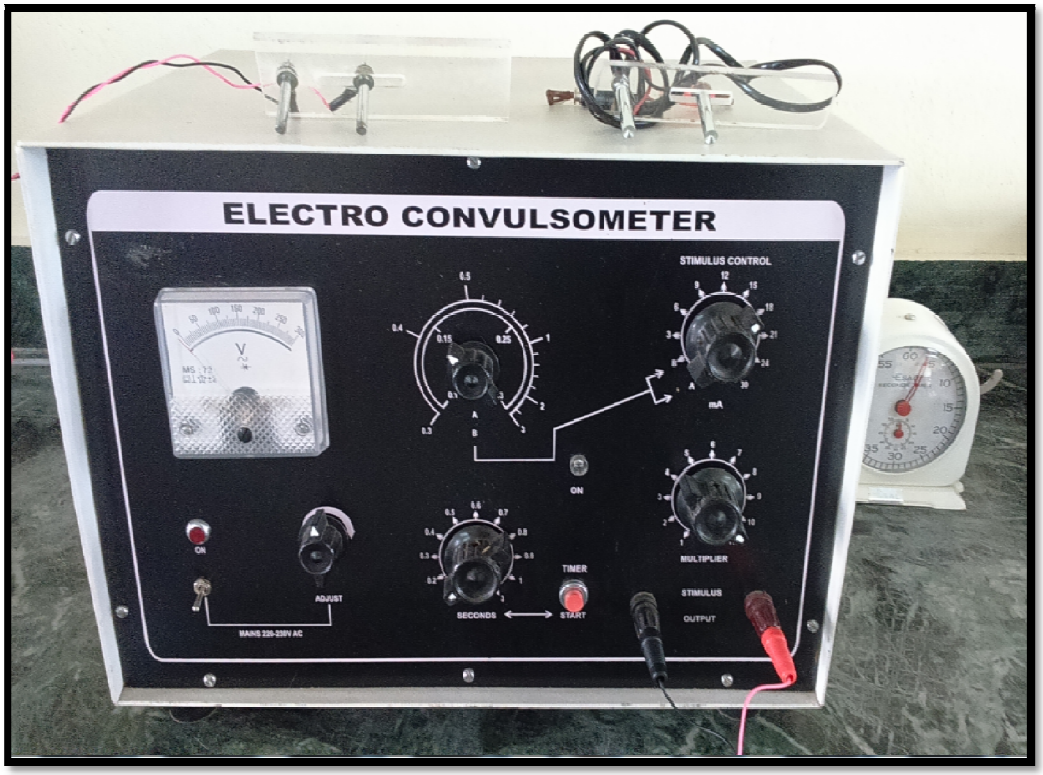
Annexure 3: Ethanolic extract of *Elettaria cardamomum* seeds

Annexure 4: Drugs and chemicals used for evaluating anticonvulsant activity

Annexure 5: Drugs used for evaluating analgesic activity

Annexure 6: Electroconvulsiometer



List of abbreviations	
µA	Micro Amperes
µg	Micro Grams
µL	Micro Liters
^{o}C	Degree Celsius
ANOVA	Analysis of Variance
BW	Body Weight
CAT	Catalase
cm	Centimeters
DPPH	1,1-diphenyl-2-picryl hydrazyl
Ec.Cr	Elettaria cardamomum crude extract
g	Grams
GABA	Gamma Amino Butyric Acid
GC-MS	Gas Chromatography Mass Spectrometry
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GST	Glutathione-S-Transferase
GTCS	Generalized Tonic Clonic Seizures
HPTLC	High Performance Thin layer Liquid Chromatography
Hz	Hertz
i.p.	Intraperitoneally
Kg	Kilo Grams
L	Liters
mA	Milliamperes
MDA	Malondialdehyde
MES	Maximal Electroshock induced Seizures
mg	Milligrams
MIC	Minimum Inhibitory Concentration
mL	Milliliters
mm	Millimeters
mV	Millivolts
NSAIDs	Non steroidal Anti-Inflammatory Agents
PTZ	Pentylenetetrazole
ROS	Reactive Oxygen Species
s.c	Subcutaneous
SD	Standard Deviation
SOD	Superoxide Dismutase
w/v	Weight/Volume